

ANNEX B

Original version August 2007, revised in August 2010

Metam-sodium

B.9 Ecotoxicology

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.9.0 Introductory note [added in August 2010]

This document is based on the updated version of Vol.3 B.9 of the DAR, which was peer-reviewed in July 2008 (PRAPeR 53). Evaluations presented in addendum to B.9, dated June 2008, which were peer-reviewed and accepted by PRAPeR 53 (July 2008), have been incorporated in this document as well, for the sake of completeness. Also, the outcome of PRAPeR 53 which was presented in the updated DAR, dated August 2008, was included in this document. Finally, evaluations of new data that were provided in the resubmission dossier (2010) have been added and are highlighted in yellow.

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

B.9.1.1 Acute oral toxicity (Annex IIA 8.1.1)

Avian Single Dose Oral LD₅₀ Metam-sodium (Aqueous solution) to the Bobwhite Quail. (Munk R., 1985).

Guidelines :

EPA 71-1 : Avian single-dose oral LD₅₀

GLP :

No, study was performed prior to GLP implementation

Material and Methods :

Test substance : metam-sodium, 42.2 % aqueous solution, batch n° : ZH 130 585

Test species : *Colinus virginianus* (bobwhite quail)

Sex, weight, age : 5 male and 5 female birds per pen, 1 pen for the control and per treatment, mean weight at start : 165.4 – 172.1 g (males) and 164.7 – 169.4 g (females), about 4 - 5 months old at start

Applied concentrations :

nominal : control (carrier control bidistilled water); 125, 250, 500, 1000, 2000 mg/kg b.w.

measured : 105.6 % of nominal concentrations

Type of application : oral administration by gavage into the crop (10 mL test solution/kg b.w.)

Time of exposure : one single application, monitoring during 14 days

Test conditions :

temperature : 21 °C

relative humidity : 50 – 60 %

photoperiod : 16/8 hours light/dark cycle

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 125 and 250 mg/kg b.w. Mortality at the treatment levels of 500, 1000 and 2000 mg/kg b.w. was 50 %, 100 % and 100 % respectively.

Clinical signs : In the control group and at the treatment level of 125 mg/kg b.w., all birds were in good health during the whole study. At the treatment levels of 250, 500, 1000 and 2000 mg/kg b.w., severe toxic signs such as apathy, prone and side position, ruffled feathers, difficulties in walking (ataxia), hanging wings and diarrhoea were seen, in principle increasing in severity and duration with the increase in the doses. Generally at the treatment levels of 1000 and 2000 mg/kg b.w., the toxic signs could be diagnosed up to death; at the treatment levels of 250 and 500 mg/kg b.w., all survivors had completely recovered from day 12 onward.

Macroscopic post mortem examination : Macroscopic examination in the surviving birds at the treatment level of 125 mg/kg b.w. revealed a slightly-thickened crop wall, fused with the covering skin and at the treatment levels of 250 and 500 mg/kg b.w., severe thickening of the crop wall, fused with the skin was observed.

Macroscopic examination of the birds that died at the treatment levels of 500, 1000 and 2000 mg/kg b.w. revealed general congestive hyperemia. In one female of the 1000 mg/kg b.w. treatment group, severe emaciation was observed.

Body weight : Beginning at the treatment level of 250 mg/kg b.w., there was a dose-related reduction in the mean body weight at the end of the study. The reduction was more pronounced in the male than in the female birds, but was not statistically significant. A significant reduction in body weight at the treatment level of 500 mg/kg b.w. was observed in the male and female survivors.

Feed consumption : The feed consumption at the treatment level of 125 mg/kg b.w. was in the same order of magnitude as the control group. At the higher treatment levels of 250, 500 and 1000 mg/kg b.w., there was a compound and dose-related reduction in the mean feed consumption. At the treatment level of 2000 mg/kg b.w.,

no feed uptake at all could be measured since all birds died. By the beginning of the second week of the study the feed uptake of the male and the female birds at the treatment level of 250 and 500 mg/kg b.w. had returned to normal.

Conclusions :

The study is acceptable.

Endpoints :

LD₅₀ (*Colinus virginianus*) = 500 mg/kg b.w. = 211 mg a.s./kg b.w.

NOEL (*Colinus virginianus*) < 125 mg/kg b.w. = 52.75 mg a.s./kg b.w.

Added in August 2010:

Acute Oral Toxicity (LD₅₀) of Methyl Isothiocyanate to Mallard Duck. (Ross D.B., Cameron M.M., Roberts N.L., 1977).

The acute oral toxicity (LD₅₀) of Methyl Isothiocyanate to the mallard duck was tested according to E.P.A. Guidelines. The study was reviewed by Dennis J. McLane, a wildlife biologist.

The ducks vomited the test material. Based on this the study is not scientifically sound and does not meet the guideline criteria for an oral LD₅₀.

A summary submitted in connection with list B procedure resulted in the review of this study.

Guidelines :

EPA 71-1 : Avian single-dose oral LD₅₀

GLP :

Not reported

Material and Methods :

Test substance : MITC, chemical purity : not reported, batch n° : not reported

Test species : *Anas platyrhynchos* (mallard duck), 10 organisms per treatment level

Sex, weight, age : not reported

Applied concentrations :

nominal : control (10 mL corn oil); 12, 24, 42, 72, 143, 188 mg/kg b.w.

Type of application : oral administration by gavage into the crop

Time of exposure : one single application, monitoring during 14 days

Test conditions :

temperature : not reported

relative humidity : not reported

photoperiod : not reported

Findings :

Mortality : No mortalities occurred in the control or at the two lowest treatment levels of 12 and 24 mg/kg b.w. In the treatment groups of 42, 72, 143 and 188 mg/kg b.w. 2, 1, 3 and 9 birds died respectively.

Gross pathology : No abnormalities were observed either in mortalities or in these birds which survived to the determination of the study.

Bird health : After dosing with test compound, all ducks vomited within about five minutes, except birds in the treatment groups of 12 and 24 mg/kg b.w. which attempted to vomit, not all succeeding. Birds in the treatment groups of 72, 143 and 188 defecated often, and lay with their eyes closed, panting vigorously. Birds in the treatment groups of 143 and 188 mg/kg b.w. became very weak and lethargic.

After 3 hours two very weak ducks (no.s 144 and 165) were found to have slight constriction of the pupils. No. 144 died shortly afterwards, No. 165 died within the next 10 hours.

Surviving birds had recovered in 2-3 days, and appeared to remain in good health until termination of the study.

Body weights : The majority of the ducks lost weight during the first week after treatment. As the controls also showed this loss in body weight, this was not considered to be a result of the administration of the test compound.

Food consumption : It was not possible to record feed consumption as the mallard duck is a very untidy feeder and a considerable amount of spillage occurred.

Conclusions of the study authors :

LD_{50} (*Anas platyrhynchos*) = 136 (79 – 235) mg/kg b.w.

As the ducks vomited shortly after dosing the amount of dose released was not known. Consequently the above values are approximations of doses administered.

Discussion and interpretation of the study results by the reviewer :

The following items were not reported :

- percent active ingredient
- age of the ducks
- length of the acclimation period
- ducks of the same hatch
- day but not time of death
- food consumption
- health / physical condition of birds prior to testing
- the method of assigning birds to pens
- the size of the pens
- photoperiod or ducks reproductive condition

The following items do not follow guideline criteria :

- the dose were not geometrically spaced
- the birds vomited the test substance

Conclusions of the reviewer :

The study is invalid. Due to the regurgitation of the test material, the test does not show how much material was administered at each test level.

Conclusions of the RMS :

RMS agrees with the above statement of the reviewer. Indeed, the study cannot be considered acceptable due to the regurgitation of the test substance.

However, a valid endpoint is obtained from the study with metam-sodium: LD_{50} (*Colinus virginianus*) = 500 mg/kg b.w. = 211 mg a.s./kg b.w.

As metam is rapidly transformed to MITC, RMS has considered that it is possible to derive a surrogate LD_{50} of MITC based on the endpoint with metam and considering a 1/1 molar transformation (molecular ratio of 0.566). The corresponding endpoint for MITC is LD_{50} = 119 mg/kg b.w. This endpoint will be used in the avian risk assessment and it is in close agreement with the endpoint from the study with MITC.

B.9.1.2 Avian dietary toxicity (5 day) (Annex IIA 8.1.2)

Metam Concentrate: 8-Day Acute Dietary LC_{50} Study in Bobwhite Quail. (Pedersen C.A., Solatycki A.M., 1993a).

Guidelines :

EPA 71-2 : Acute Dietary LC_{50} Test for Waterfowl and Upland Game Birds

GLP :

Yes

Material and Methods :

Test substance : metam-sodium, 43 % aqueous solution, batch n° : 650

Test species : *Colinus virginianus* (bobwhite quail)

Sex, weight, age : 10 birds per pen, 5 pens for the control, 1 pen per treatment, not sexed, mean weight at start : 26 – 32 g, 14 days old at start

Applied concentrations :

nominal : control; 312, 625, 1250, 2500, 5000 mg a.s./kg feed

Type of application : dietary application

Time of exposure : short-term feeding test (5-day exposure period, 3-day recovery period)

Test conditions :

temperature : 23 °C (exposure and recovery period)
relative humidity : 48 % (exposure period), 54 % (recovery period)
photoperiod : 24 hours light per day

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 312, 625, 1250 and 2500 mg a.s./kg feed. Mortality at the highest treatment level of 5000 mg a.s./kg feed was 10 %.

Clinical signs : On test day 5, one bird at the highest treatment level of 5000 mg a.s./kg feed was found dead and the other birds in the treatment group were moving slowly. Complete remission of all clinical signs was achieved in the survivors by test day 6.

Macroscopic post mortem examination : No abnormal findings were observed in the bird that died at the highest treatment level of 5000 mg a.s./kg feed. No treatment-related pathological findings were observed in 24 arbitrarily selected surviving birds from all treatment levels and the control group.

Body weight : Reduced body weights were noted on test day 8 at the treatment levels of 625, 1250, 2500 and 5000 mg a.s./kg feed.

Feed consumption : Reduced feed consumption was recorded during test days 1 through 5 at the treatment levels of 625, 1250, 2500 and 5000 mg a.s./kg feed. Reduced feed consumption was recorded during test days 6 through 8 only at the highest treatment level of 5000 mg a.s./kg feed.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Colinus virginianus*, 5 d) > 5000 mg a.s./kg feed or 448 mg a.s./kg b.w./day based on a mean body weight of 33.5 g/bird and a mean feed consumption of 3 g/bird/day

NOEL (*Colinus virginianus*, 5 d) = 312 mg a.s./kg feed or 56.7 mg a.s./kg b.w./day based on a mean body weight of 44 g/bird and a mean feed consumption of 8 g/bird/day

Metam Concentrate: 10-Day Acute Dietary LC₅₀ Study in Mallard Ducklings. (Pedersen C.A., Solatycki A.M., 1993b).

Guidelines :

EPA 71-2 : Acute Dietary LC₅₀ Test for Waterfowl and Upland Game Birds

GLP :

Yes

Material and Methods :

Test substance : metam-sodium, 43 % aqueous solution, batch n° : 650

Test species : *Anas platyrhynchos* (mallard duck)

Sex, weight, age : 10 birds per pen, 3 pens for the control, 1 pen per treatment, not sexed, mean weight at start : 77 – 94 g, 7 days old at start

Applied concentrations :

nominal : control; 312, 625, 1250, 2500, 5000 mg a.s./kg feed

Type of application : dietary application

Time of exposure : short-term feeding test (5-day exposure period, 5-day recovery period)

Because deaths occurred on day 6 of the study, which was the first day of the normal 3-day recovery period, the recovery was extended an additional two days.

Test conditions :

temperature : 22 °C (exposure and recovery period)

relative humidity : 62 % (exposure period), 60 % (recovery period)

photoperiod : 24 hours light per day

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 312, 625, 1250 and 2500 mg a.s./kg feed. Mortality at the highest treatment level of 5000 mg a.s./kg feed was 10 %.

Clinical signs : No signs of toxicity were noted in the control groups or at the treatment levels of 312, 625 and 1250 mg a.s./kg feed throughout the investigation. Clinical signs noted at the treatment level of 2500 mg a.s./kg feed include inactivity, moving only when gently prodded and crawling when attempting to walk. The birds at the highest treatment level of 5000 mg a.s./kg feed exhibited clinical signs including instability, inactivity,

crawling when attempting to walk, hunching in a corner, moving slowly, huddling and death. Complete remission of all clinical signs was achieved in survivors by study termination (test day 10).

Macroscopic post mortem examination : The one bird that died at the highest treatment level of 5000 mg a.s./kg feed had gaseous intestines and a dark red-coloured liver. No treatment-related pathological findings were observed in 24 arbitrarily selected surviving birds from all treatment levels and the control group.

Body weight : Reduced body weights were noted on test day 10 at the treatment levels of 625, 1250, 2500 and 5000 mg a.s./kg feed.

Feed consumption : Reduced feed consumption was recorded during test days 1 through 5 at the treatment levels of 312, 625, 1250, 2500 and 5000 mg a.s./kg feed and during test days 6 through 10 at the treatment levels of 625, 1250, 2500 and 5000 mg a.s./kg feed.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Anas platyrhynchos*, 5 d) > 5000 mg a.s./kg feed or 324 mg a.s./kg b.w./day based on a mean body weight of 139 g/bird and a mean feed consumption of 9 g/bird/day

B.9.1.3 Subchronic and reproductive toxicity (Annex IIA 8.1.3)

Not required. Metam-sodium is applied once by soil injection. Therefore continued or repeated exposure of adults or nest sites during the breeding season is unlikely to occur.

B.9.1.4 Acute oral toxicity of the preparations (Annex IIIA 10.1.1)

Not required.

B.9.1.5 Supervised cage or field trials (Annex IIIA 10.1.2)

Added in August 2010:

Bird censuses on sterilized and unsterilized carrot plots in Les Landes, France during spring 2008. (Noordam A., Bakker F., 2010).

Aim

The aim of this study was to propose a list of bird species in treated and untreated carrot field that can be addressed as focal bird species in a refined risk assessment for plant protection products.

Objectives

The objectives of this study were to determine whether the presence of bird species and their frequency is related to the metam sodium treatment, both in time (before and after treatment), as in space (comparing control, reference and test item treated plots).

Guidelines :

Not applicable

GLP :

Not applicable

Material and Methods :

Study area :

The experimental site was a 12 ha field in Les Landes (France), which is a major area for carrot production. Within this area, 8 square plots of 1 ha each and 4 more rectangular plots with 1 rounded side were established.

Method and parameters :

Two of these plots were not cultivated at all. The remaining plots were tilled on 3 April 2008, subjected to injection on 8 and 9 April 2008, ploughed on 29 April 2008 (including butt formation) and subsequently sown with carrot on May 5th, 2008. In four of these plots injection was with metam-sodium. The other six were injected with water only. Two of the water plots were subsequently treated with a broad spectrum insecticide, registered for sterilization (Pyrinex, a.i. chlorpyrifos).

Several methods were started simultaneously to compare their effectiveness: 1) continuous video-recording with analysis of the recordings afterwards; 2) continuously over viewing the field from one high central point; 3) scoring the birds during a standardized walk through all the plots.

Bird censusing was performed during two critical periods in the spring of 2008. A total of 23 observation rounds was made for 1 to 8 days after application of the sterilant, and a further 17 observations rounds 11 to 16 days after sowing. During the study period there was little or no vegetation on the land.

Data recording and analysis :

Evaluation of the used methods:

1) The video-recording missed nearly all birds that were simultaneously noted with method 2; 2) Over viewing the field from one high central point proved more effective, although small birds on the soil at the other end of the field were regularly overlooked. Also due to the relief of the plots, some plots were more or less visible from the observation point;

3) The standardized walk through all the plots proved to yield the most informative and systematic data which has been used as basis for this report.

Findings :

A total of 74 bird species were recorded during the observations in April and May 2008. However, only 15 species were actually seen on or above the field, some trying to feed there: Kestrel (*Falco tinnunculus*), Barn Swallow (*Hirundo rustica*), Meadow Pipit (*Anthus pratensis*), White Wagtail (*Motacilla alba*), Yellow Wagtail (*Motacilla flava*), Black Redstart (*Phoenicurus ochruros*), Mistle Thrush (*Turdus viscivorus*), Starling (*Sturnus vulgaris*), Carrion Crow (*Corvus corone*), Chaffinch (*Fringilla coelebs*), Yellowhammer (*Emberiza citrinella*), Reed Bunting (*Emberiza schoeniclus*), Serin (*Serinus serinus*), Greenfinch (*Carduelis chloris*), Goldfinch (*Carduelis carduelis*).

The most commonly observed species were the barn swallow in May and the yellowhammer in April. This was both in terms of the number of observations and in numbers of individuals. The second most frequently observed species in both periods was the white wagtail. The meadow pipit, only observed in April was the second most numerous species in the field, followed by starling, carrion crow, white wagtail and black redstart that were seen on both occasions.

Table B.9.1.5-1 : Summary of numbers of individuals observed in the 12 plots of the experimental carrot field near Gabarret in April 2008. WATER = injected with water; METAM = metam-sodium treated plots; CPF = chlorpyrifos treated plots; UNCULT. = plots not cultivated at all

10-16 APRIL 2008 (24 OBSERVATIONS)					
Species	TOTAL	WATER	METAM	CPF	UNCULT.
Kestrel	2	0	1	0	1
Barn Swallow	9	1	2	0	6
Meadow Pipit	94	0	16	0	78
White Wagtail	17	12	2	0	3
Yellow Wagtail	11	0	1	0	10
Black Redstart	3	0	1	1	1
Mistle Thrush	5	0	2	2	1
Starling	23	0	17	4	2
Carrion Crow	20	10	5	4	1
Chaffinch	14	4	3	3	4
Yellowhammer	355	89	116	57	93
Reed Bunting	3	0	0	2	1
Serin	0	0	0	0	0
Greenfinch	0	0	0	0	0
Goldfinch	0	0	0	0	0
SUM	556	116	166	73	201
PER PLOT	46	29	42	37	101

Table B.9.1.5-2 : Summary of numbers of individuals observed in the 12 plots of the experimental carrot field near Gabarret in May 2008. WATER = injected with water; METAM = metam-sodium treated plots; CPF = chlorpyrifos treated plots; UNCULT. = plots not cultivated at all

16-20 MAY 2008 (17 OBSERVATIONS)					
Species	TOTAL	WATER	METAM	CPF	UNCULT.
Kestrel	0	0	0	0	0
Barn Swallow	156	62	35	11	48
Meadow Pipit	0	0	0	0	0
White Wagtail	22	17	3	2	0
Yellow Wagtail	1	1	0	0	0
Black Redstart	12	7	1	1	3
Mistle Thrush	0	0	0	0	0
Starling	31	8	3	14	6
Carrion Crow	10	1	2	0	7
Chaffinch	0	0	0	0	0
Yellowhammer	0	0	0	0	0
Reed Bunting	0	0	0	0	0
Serín	8	0	0	0	8
Greenfinch	2	0	0	0	2
Goldfinch	8	0	0	0	8
SUM	250	96	44	28	82
PER PLOT	21	24	11	14	41

The distribution of birds over the experimental site depended on several factors: the species, the cultivation history, the treatment and the time after treatment. In April around two times more birds were seen than in May. On both occasions significantly more birds were seen in the uncultivated plots. However, whereas in April the lowest number of birds was seen on the water plots, the situation was reversed in May when the water plots had the highest numbers. In both cases, the dependence of bird frequency on treatment was highly significant. This is most likely a consequence of differential food availability among treatments. In April, when the soil was recently treated, there were probably more easy-to-capture food items on the surface of the plots having received an insecticidal treatment than on the water plots. In May this situation was reversed. The water plots had higher densities of food items for in particular insectivorous birds than the insecticide treated plots.

In this (indirect) response to treatment there were however clear differences among species. The most numerous bird in April, the Yellowhammer, was distributed over the cultivated part of the field independent of treatment. The Meadow Pipits were observed 6 times on the uncultivated eastern plot and 4 times on metam-sodium treated plots, but never in the other treatments. Their large abundance is mainly due to one observation day when a numerous migratory troop was present (73 individuals on 10 April 2008). Of the 23 Starlings observed during the April observations, 17 occurred in metam-sodium treated plots and 4 in chlorpyrifos treated plots. None was seen in the water plots and only 2 in the uncultivated plots. In May Starlings were most numerous in the chlorpyrifos treated plots, otherwise they were seen more often in the water plots than in the metam-sodium plots. The White Wagtail was seen mostly on the cultivated land, with a very strong preference for the water plots. Most observations relate to one pair of Wagtails that was resident to the field. Barn swallows were infrequently observed in April, but dominated the observations in May. They were observed in the highest density (specimens/ha) over the uncultivated land, but when they foraged over the field, they were most frequently seen over the water plots.

In April Carrion Crows were mainly observed on the cultivated part of the field, whereas in May most individuals were seen in the uncultivated plots. It is unclear what caused this switch.

The Mistle Thrush was not a frequent visitor to the field and was only observed in the field in April. In May it was never seen on the field itself although regularly a Mistle Thrush was flying high over. The species is worth attention, however, as it exhibited a clear increase in foraging activity after the soil sterilization. Especially on April 11th and somewhat less so on the next day two Mistle Thrushes were nearly continuously present in a metam-sodium treated plot, where they were collecting the massively emerging earthworms. They were regularly seen taking off from this plot with one or more earthworms in their beaks leaving in northern direction.

Conclusions :

With a few exceptions the bare cultivated land was definitely not a favoured habitat for most of the 74 bird species identified in and around the field and the frequency of bird visits remained low throughout the study period. Starling and Mistle thrush were observed to remove food items from the treated soil and consequently may have been exposed to contaminated food. In April Crows were regularly seen on the field, but to what extent they may have been exposed is unclear. Their shyness prevented detailed observations. The White wagtail was resident to the field but somehow restricted its presence to water treated plots. Swallows foraged low over the field, obviously eating insects emerging from the soil. To what extent these insects may have been contaminated is unknown.

Conclusions of the RMS :

The study is considered acceptable and is taken into account in the avian risk assessment.

B.9.1.6 Acceptance of bait, granules or treated seeds by birds (palatability test) (Annex IIIA 10.1.3)

Not required.

B.9.1.7 Effects of secondary poisoning (Annex IIIA 10.1.4)

Added in August 2010:

The level of MITC in arthropods sampled in a field treated with metam-sodium was determined analytically.

METAM SODIUM: A field study to determine residues in carrot field invertebrates as potential food items for birds and small mammals in SW France. (Bakker F. & Roig J., 2010).

Guidelines :

Wolf C. and Schneider K. (2007)

GLP :

Yes

Material and Methods :

Test substance : Nemastol 510, formulation containing nominally 510 g metam-sodium/L (analytically 499 g a.s./L), batch n°: G080311261

Test species : soil and surface dwelling arthropods, e.g. beetles and spiders

Sampling methods : pitfall trap sampling

Collected organisms : Post-application samples were taken exclusively from the metam-sodium treated plots (1 x 1200 L Metam Sodium/ha, equivalent to 612 kg a.s./ha) 1, 2, 3, 4, 5, 6, 7, 9, 10, 14, 15, 19, 20, 29 and 30 days after application (beginning of April until beginning of May). Only the samples collected 24, 48 and 72 hours after treatment were analysed for MITC. A pre-application sample (end of March) was collected to obtain a residue-free matrix.

The specimens were further divided into categories representing potential food items: Coleoptera, Spiders, Millipedes and others, in which case the number of individuals and frozen weight of each category was recorded.

Type of test : field study

Test substrate : Poor sandy soil with low content of organic matter, on a commercial carrot field near Gabarret and Escalans, Les Landes, (France): 93.7 % sand ($0.05 < D < 2$ mm), 1.8 % coarse silt ($0.02 < D < 0.05$ mm), 1.8 % fine silt ($0.002 < D < 0.02$ mm), 2.7 % clay ($D < 0.002$ mm), 1.04 % organic matter, $< 3\%$ CO_3^{2-} , pH : 7.3.

Field description :

The experimental field was flanked on the North-West side by forest, on the South-West side by maize, and grassland on the other sides.

The experiment started on bare soil. After injection of the test item the soil must be sealed for 3 weeks, after this the soil is ploughed to release the test item from the soil. The last population sample was taken 4 week after the ploughing. Two weeks after the ploughing the carrot seeds (*Daucus carota*) were sown, this is one week after the last sampling. Thus the whole experimental period was conducted on bare soil and no crop phenological stages according to the BBCH-scale were recorded.

Applied concentrations :

The ground dwelling arthropods were collected following the application of control (1200 L water /ha); and test item (1 x 1200 L Metam Sodium/ha, equivalent to 612 kg a.s./ha).

The test item was applied in accordance with GAP use, i.e. a single application at the rate mentioned. The application took place on 7 (2 plots) and 8 (2 remaining plots) April 2008. METAM-SODIUM was injected into the soil, immediately followed by sealing of the ground. Three weeks later the soil was ploughed in order to dispose of volatile chemical residues. The water control was applied by treating the soil in a similar way as the test item, but with water. In this way potential insecticidal properties of METAM-SODIUM could be directly compared to the control without the confounding effects of the application procedure.

Exposure route :

Plots were situated in an area of approximately 12.5 ha. There was an overhead irrigation system, which dictated the semi-circular geometry of the field. Inside this field 10 plots were established. Water control and METAM-SODIUM treated plots (4 plots) measured approximately 1 ha.

All plots were treated entirely. There were no untreated areas between the plots.

Sampling :

All samples were taken from the central 10 x 10 m area of each plot. Soil samples were taken on five random positions within the 10 x 10 m sampling area of each plot. Two groups of five pitfall traps were established in the central area of each plot, using two mirroring W-configurations. All traps were removed and reinstalled for ploughing, harvesting the carrots, and sowing of the maize (using GPS information)

Test conditions : average meteorological conditions

Residue analysis : MITC is distilled from water into a cooled reservoir containing ethyl acetate. The aqueous distillate is saturated with sodium chloride and partitioned into ethyl acetate. Analysis is performed using gas chromatography-mass spectrometry.

Findings :

The recovery from arthropods fortified with MITC is given below. Residues in the control sample were shown to be less than the 0.05 mg/kg LOQ.

The levels of MITC measured in all of the field trial samples were high compared to the original method validation levels (0.05 and 0.5 mg/kg) and analytical sample 2 gave a response above the highest calibration point included in the batch (at 10 mg/kg equivalence). MITC residues in test samples were estimated to be in the range 3.5 to 13.9 mg/kg.

Table B.9.1.7-1 : MITC residues in arthropod samples

Sample number	description	MITC residue (mg/kg)	Fortification level (mg/kg)	Recovery (%)	Control residue (mg/kg)
Analytical sample 1	24 h after treat (plots 3 & 5)	4.0	0.05	109.5	ND
Analytical sample 2	48 h after treat (plots 1 & 7)	13.3*	0.05	109.5	ND
Analytical sample 3	72 h after treat (plot 1)	5.3	0.05	109.5	ND
Analytical sample 4	72 h after treat (plot 7)	6.3	0.05	109.5	ND
Analytical sample 5	72 h after treat (plot 3)	3.5	0.05	109.5	ND
Analytical sample 6	72 h after treat (plot 5)	4.2	0.05	109.5	ND
Analytical sample 7	9 d before treat (plot 2)	<0.05**	0.05	109.5	ND

*1g (±0.02g) subsample used for each sample apart from analytical sample 2 where a 0.95g subsample was taken. No correction has been made for the subsample size.

**0.05 mg/kg = LOQ

ND = Not Detected subject to 0.05 mg/kg LOQ

Conclusions :

The data presented demonstrate that MITC can successfully be determined in arthropods. The residues were in the range of 3.5 mg/kg to 13.3 mg/kg, during first 72 hours after application with the test item.

Conclusions of the RMS :

The study is considered acceptable and is taken into account in the avian risk assessment.

B.9.1.8 Summary of effects on birds – exposure and risk assessment for birds (Annex IIIA 10.1)**Revised in August 2010:**

Table B.9.1.8-1 : Summary of effects of metam-sodium on birds

Test species	Test substance	Test system	Endpoints	References
<i>Colinus virginianus</i>	metam-sodium	acute oral toxicity	LD ₅₀ = 211 mg a.s./kg b.w.	Munk R., 1985
<i>Colinus virginianus</i>	metam-sodium	dietary toxicity	LC ₅₀ > 448 mg a.s./kg b.w./day	Pedersen C.A., Solatycki A.M., 1993a
<i>Anas platyrhynchos</i>	metam-sodium		LC ₅₀ > 324 mg a.s./kg b.w./day	Pedersen C.A., Solatycki A.M., 1993b

First tier risk assessment for birds :

The risk assessment for birds is based on the Guidance Document for birds and mammals under Council Directive 91/414/EEC of November 2002. As a worst case it was assumed that the birds obtained 100 % of their diet in the treated area.

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil; the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is compressed with a roller. After a waiting period of 2 to 4 weeks, the soil is cultivated to allow the non-mineralised gasses to disperse. Since the product is phytotoxic, it is advisable to perform a germination test (Cress-test) on samples of soils collected from the treated field, before planting. Birds are not exposed to the uses in greenhouse.

1 - Risk assessment presented by the notifier:**Acute risk assessment of Metam-Sodium on birds.**

EFSA have requested an acute risk assessment for metam-sodium for insectivorous birds that might feed on freshly treated bare soil fields.

Based on the physico-chemical characteristics and toxicological endpoints of metam sodium it is not meaningful to calculate the risk assessment for the parent active substance but instead for the primary metabolite MITC.

The degradation rate of metam-sodium was assessed in four different soil types, the mean DT₅₀ at 20°C was 10 minutes (minimum - maximum DT₅₀ = 4 – 17 minutes), whereas the DT₉₀ is set to be 34 minutes (minimum - maximum DT₉₀ = 13 – 57 minutes). MITC is the only relevant metabolite of metam-sodium (Hall, B.E. *et al.* 2004).

The toxicity of metam-sodium and MITC to birds has been assessed in acute toxicity tests. The relevant toxicological endpoints of metam-sodium and MITC are presented in the table below. Comparing these endpoints the acute toxicity of MITC is higher to birds than metam-sodium (see Table B.9.1.8-2).

Table B.9.1.8-2: The relevant toxicological endpoints of metam-sodium and MITC for birds

	Metam-Sodium (mg/kg bw/day)	References	MITC (mg/kg bw/day)	References
Acute avian LD ₅₀	<i>Colinus virginianus</i> (Bobwhite quail) 211	Munk, 1985	<i>Anas platyrhynchos</i> (Mallard duck) 136	Ross <i>et al.</i> , 1977a

Conclusion:

Based on the very fast degradation of metam-sodium into the more toxic MITC, we consider that when the acute risk of MITC is acceptable for birds, the risk of metam-sodium will also be acceptable.

Acute risk assessment of MITC on birds.

The Acute Risks to Birds and Mammals potentially exposed following proposed commercial use of Metam Sodium. (Brown K.C., 2010) Unpublished report

Summary:

Glasshouse uses of metam sodium are excluded from this risk assessment since there will be no exposure to birds from closed crop systems. The field uses which distribute MITC widely throughout the soil, injection/rotavation, injection/coverage and drip irrigation are considered to present the greatest risk to birds, since the majority of the soil dwelling invertebrates will receive almost uniform exposure. Following a field survey, a risk assessment for birds feeding on earthworms is also provided since such species could also be at risk.

Acute toxicity of MITC to birds : LD₅₀ = 136 mg/kg bw/d (Ross *et al.* 1977)

Selection of focal species

A field survey of birds feeding on invertebrates of bare soil treated with metam-sodium (Noordam and Bakker, 2010) recorded observations of two bird species seen feeding in bare fields (both treated and control). For this risk assessment two focal species will be considered, the starling and the mistle thrush.

Measured Residues in Insects

Specimens of invertebrates were collected from a field study with metam sodium applied at 612 kg as/ha (the rate for strawberries and tomato) in preparation for sowing (Bakker and Roig, 2010) and analysed for MITC residues. The equivalent dose rate of MITC in this study was 346 kg MITC/ha. An initial residue mean value of 7.3 mg/kg was derived.

Analysis of the RUD values in the EFSA (2009) guidance for generic focal species of bird in bare soil show that the 90th centile is between 1.85 and 2.17 times the mean RUD value. In the absence of sufficient information to reliably define the distribution about the mean for the initial residue results it is proposed to use a multiplier of 2.17 so as to estimate a worst case 90th centile for the residue in insects of 16.0 mg/kg at a metam sodium application rate equivalent to 346 kg MITC/ha (resulting in an RUD (Residue per unit Dose) value of 0.046 mg/kg at a rate of 1 kg MITC/ha).

Estimated Residues in Earthworms

The BCF formula in SANCO/4145/2000 is not considered to be appropriate due to the extremely short half life of MITC of 2.1 days. The use of BCF assumes residues are acquired over a longer period of time than would be appropriate for this risk assessment.

As residue data on earthworms are not available it is proposed to use the measured fresh residues for invertebrates as a surrogate.

Risk Assessment for Insectivorous Birds

Starling has been selected as the appropriate focal species of insectivore for bare soil treated with metam sodium. Starling has a body weight of 79.9 g for females and 84.7 g for males (Buxton *et al.*, 1998). Considering the mean body weight of 82.3 g, daily consumption and energy expenditure for a passerine feeding entirely on soil invertebrates has been calculated using the PSD (now CRD) dietary calculation as being 102.2 g fresh food per bird per day with a Daily Energy Expenditure of 223.35 KJ/animal.

FIR/bw is calculated from mean body weight and daily dietary consumption as being 1.24 g/g bw

$$ETE = (FIR/bw) * C * AV * PT * PD$$

It is assumed at the first tier that starlings are obtaining all their food from the treated area. AV, PT and PD are therefore set at 1 for the first tier of this risk assessment.

The lowest acute LD₅₀ value for MITC is 136 mg/kg (mallard duck) from Table B.9.1.8-2.

Table B.9.1.8-3: Acute risk assessment for insectivorous birds in field crops

Field uses of metam sodium	MITC rate assuming complete conversion from metam sodium* (kg as/ha)	RUD	FIR/bw Starling	ETE	TER
Carrots	230 (408)	0.046	1.24	13.1	10.4
Corn Salad	173 (306)	0.046	1.24	9.9	13.7
Potato	86.3 (153)	0.046	1.24	4.9	27.7

*metam sodium application rate in brackets

The TER values derived for use in carrots, corn salad and potatoes are above the threshold of 10 and indicate acceptable acute risk to insectivorous birds.

Risk Assessment for Birds feeding on earthworms

Mistle thrush has been selected as the appropriate focal species of worm feeding bird for bare soil treated with metam sodium.

Mistle thrush has a mean body weight of 115 g (Buxton *et al.*, 1998). The daily consumption for a 115 g passerine feeding entirely on earthworms has been estimated as 127 g using the FIR/bw figure of 1.1 for worm feeding birds in SANCO 4145/2000.

$$ETE = (FIR/bw) * C * AV * PT * PD$$

It is assumed at the first tier that mistle thrush obtain all their food from the treated area.

AV, PT and PD are therefore set at 1 for the first tier of this risk assessment.

The lowest acute LD₅₀ value for MITC is 136 mg/kg (mallard duck).

Table B.9.1.8-4: Acute risk assessment for earthworm feeding birds in field crops

Field uses of metam sodium	MITC rate assuming complete conversion from metam sodium* (kg as/ha)	RUD	FIR/bw Mistle Thrush	ETE	TER
Carrots	230 (408)	0.046	1.1	11.6	11.7
Corn Salad	173 (306)	0.046	1.1	8.75	15.5
Potato	86.3 (153)	0.046	1.1	4.3	31.6

* metam sodium application rate in brackets

The TER values derived for use in carrots, corn salad and potatoes are above the threshold of 10 and therefore demonstrate acceptable acute risk to worm feeding birds. In field grown strawberries and tomatoes injection occurs without rotavation and there is a physical barrier (of plastic or topsoil) which will prevent birds from being exposed. Very low exposure would be anticipated resulting in acceptable risk for strawberries and tomatoes.

Short-term risk assessment of MITC on birds.

Since it is assumed that in the course of some days, birds will not always be exposed to insects contaminated with MITC but also to uncontaminated food, the notifier proposed the short-term risk assessment also as relevant.

More detailed calculations performed by RMS are presented below.

2 - Risk assessment performed by RMS:

Acute and short-term toxicity endpoint MITC:

RMS disagrees with the notifier on the choice of the acute toxicity endpoint to be used in the avian risk assessment.

The study "Acute Oral Toxicity (LD₅₀) of Methyl Isothiocyanate to Mallard Duck. (Ross D.B. *et al.*, 1977) cannot be considered acceptable due to the regurgitation of the test substance.

However, a valid endpoint is obtained from the study with metam-sodium: LD₅₀ (*Colinus virginianus*) = 500 mg/kg b.w. = 211 mg a.s./kg b.w.

As metam is rapidly transformed to MITC, RMS has considered that it is possible to derive a surrogate LD₅₀ of MITC based on the endpoint with metam and considering a 1/1 molar transformation (molecular ratio of 0.566). The corresponding endpoint for MITC is LD₅₀ = 119 mg/kg b.w. This endpoint will be used in the avian risk assessment and it is in close agreement with the endpoint from the study with MITC.

In analogy with the acute toxicity endpoint, the RMS has considered a surrogate LC₅₀ of MITC based on the endpoint with metam (LC₅₀ (*Anas platyrhynchos*) > 324 mg a.s./kg b.w./day) and considering a 1/1 molar transformation (molecular ratio of 0.566). The corresponding endpoint for MITC is LC₅₀ > 183.4 mg/kg b.w.

Focal species:

From the field survey of birds (Noordam A., Bakker F., 2010), two focal species were identified. RMS agrees with the notifier to consider the starling and the mistle thrush for the avian risk assessment.

Measured residues in food items:

From the residue field study (Bakker F. & Roig J., 2010) residues of MITC in **invertebrates** were determined after 24, 48 and 72 hours after treatment of metam sodium at 612 kg a.s./ha (equivalent to 346 kg MITC/ha). The residue was 4.0 mg/kg (mean of 2 plots) after 24 hours, 13.3 mg/kg (mean of 2 plots) after 48 hours and 4.8 mg/kg (mean of 4 plots) after 72 hours. An initial mean residue value over 0-72 hours was calculated as 7.3 mg/kg.

For the acute risk assessment 90th percentiles are needed. From the EFSA Guidance Document on Birds and Mammals (Appendix F) mean RUD and 90th RUD are compared for ground dwelling invertebrates on bare soil. RUD 90th is 13.8 mg/kg and mean RUD is 7.5 leading to a factor 1.84. This is in agreement with the proposal of the notifier to use a factor 1.85 – 2.17. Taking the worst case factor of 2.17, the 90th residue value is calculated 16 mg/kg for an application rate of 346 kg MITC/ha. Recalculation in RUD (residue per unit dose) results in 0.046 mg/kg for an application rate of 1 kg MITC/ha.

For the short-term risk assessment the arithmetic mean residue value of 7.3 mg/kg for an application rate of 346 kg MITC/ha was used in the risk assessment. Recalculation in RUD results in 0.021 mg/kg for an application rate of 1 kg MITC/ha.

For the residue data in **earthworms** it is not appropriate to use the BCF formula, due to the extremely short half-life of MITC of 2.1 days. In absence of residue field data obtained for earthworms, RMS agrees with the notifier to use the residue values in insects as surrogate.

Risk assessment for insectivorous birds

The starling is selected as the appropriate focal species for insectivorous birds feeding on bare soil treated with metam-sodium. The body weight of starling is 84.7 g for males and 79.9 g for females, giving a mean body weight of 82.3 g (Buxton *et al.*, 1998).

$$\log(\text{DEE}) = 1.0017 + (0.7034 \times (\log 82.3)) = 2.35$$

$$\text{DEE} = 223 \text{ kJ}$$

Table B.9.1.8-5 : Calculation of the ratio of food intake rate of soil invertebrates to body weight for a starling per day

Food type	Energy content* (kJ/g dw)	Moisture content* (%)	Energy content (kJ/g ww)	Assimilation efficiency** (%)	Yield (kJ/g ww)	Fresh food intake (g/day)	FIR/bw
soil invertebrates	19.3	84.6	2.97	76	2.26	98.88	1.20

* Value taken from Table 3 in Appendix I of SANCO/4145/2000

** Value taken from Table 5 in Appendix I of SANCO/4145/2000

For the first tier calculation it is assumed that the starlings are obtaining all their food from the treated area and therefore AV, PD and PT are set at 1.

The greenhouse uses of metam-sodium are excluded from the risk assessment since there will be no exposure to birds from closed crop systems.

The use of metam-sodium in grapes at vine planting is a very localized treatment and therefore the risk will be much lower and will be covered by the other field uses.

The field uses which distribute MITC widely throughout the soil, injection/rotavation, injection/coverage and drip irrigation are considered to present the greatest risk to birds, since the majority of the soil dwelling invertebrates will receive almost uniform exposure. These crops are carrot, corn salad and potato (injection/rotavation), tomato (injection/coverage) and strawberry (drip irrigation).

Table B.9.1.8-6 : Acute risk assessment for insectivorous birds eating contaminated soil invertebrates on bare soil for the use in field crops (based on endpoint $LD_{50} = 119$ mg MITC/kg b.w.)

Field uses of metam-sodium	MITC rate assuming complete conversion from metam-sodium* (kg/ha)	FIR/bw	RUD	ETE	TER	Annex VI Trigger value
strawberry	346 (612)	1.20	0.046	19.1	6.23	10
tomato	346 (612)	1.20	0.046	19.1	6.23	10
carrot	230 (408)	1.20	0.046	12.7	9.37	10
corn salad	173 (306)	1.20	0.046	9.55	12.5	10
potato	86.3 (153)	1.20	0.046	4.76	25.0	10

* metam-sodium application rate in brackets

Based on first tier assumptions (PD, PT = 1) and initial measured residues, the acute TER for carrot is very close to the trigger value of 10. RMS considers the risk acceptable since slight refinement in the diet will lead to an acute TER higher than 10. The acute TER values for corn salad and potato indicate acceptable risk for insectivorous birds.

With strawberries the treated soil is covered with a plastic film which would be likely to deter birds and prevent them from feeding on invertebrates. Therefore RMS considers the acute risk for use in strawberry acceptable.

In field tomatoes metam-sodium is injected to a depth of 5 cm and covered with a 10 cm layer of untreated soil. However, RMS does not agree with the notifier that the layer of untreated soil would form a physical barrier preventing the birds from exposure. In the opinion of RMS risk mitigation measures are needed for field tomato.

Table B.9.1.8-7 : Short-term risk assessment for insectivorous birds eating contaminated soil invertebrates on bare soil for the use in field crops (based on endpoint $LC_{50} > 183.4$ mg MITC/kg b.w./day)

Field uses of metam-sodium	MITC rate assuming complete conversion from metam-sodium* (kg/ha)	FIR/bw	RUD	ETE	TER	Annex VI Trigger value
strawberry	346 (612)	1.20	0.021	8.72	21.0	10
tomato	346 (612)	1.20	0.021	8.72	21.0	10
carrot	230 (408)	1.20	0.021	5.80	31.6	10
corn salad	173 (306)	1.20	0.021	4.36	42.1	10
potato	86.3 (153)	1.20	0.021	2.17	84.3	10

* metam-sodium application rate in brackets

Based on first tier assumptions (PD, PT = 1) and arithmetic mean measured residues, the short-term TER values for all field uses meet the trigger value of 10 for insectivorous birds.

Risk assessment for vermivorous birds

The mistle thrush is selected as the appropriate focal species for birds feeding on earthworms on bare soil treated with metam-sodium. The body weight of mistle thrush is 115 g (Buxton *et al.*, 1998). The FIR/bw value of 1.1 was used according to SANCO 4145/2000.

For the first tier calculation it is assumed that the starlings are obtaining all their food from the treated area and therefore AV, PD and PT are set at 1.

Table B.9.1.8-8 : Acute risk assessment for vermivorous birds eating contaminated earthworms on bare soil for the use in field crops (based on endpoint LD₅₀ = 119 mg MITC/kg bw)

Field uses of metam-sodium	MITC rate assuming complete conversion from metam-sodium* (kg/ha)	FIR/bw	RUD	ETE	TER	Annex VI Trigger value
strawberry	346 (612)	1.1	0.046	17.5	6.80	10
tomato	346 (612)	1.1	0.046	17.5	6.80	10
carrot	230 (408)	1.1	0.046	11.6	10.2	10
corn salad	173 (306)	1.1	0.046	8.75	13.6	10
potato	86.3 (153)	1.1	0.046	4.37	27.3	10

* metam-sodium application rate in brackets

The acute TER values for carrot, corn salad and potato indicate acceptable risk for vermivorous birds. With strawberries the treated soil is covered with a plastic film which would be likely to deter birds and prevent them from feeding on earthworms. Therefore RMS considers the acute risk for use in strawberry acceptable. In field tomatoes metam-sodium is injected to a depth of 5 cm and covered with a 10 cm layer of untreated soil. However, RMS does not agree with the notifier that the layer of untreated soil would form a physical barrier preventing the birds from exposure. In the opinion of RMS risk mitigation measures are needed for field tomato.

Table B.9.1.8-9 : Short-term risk assessment for vermivorous birds eating contaminated earthworms on bare soil for the use in field crops (based on endpoint LC₅₀ > 183.4 mg MITC/kg b.w./day)

Field uses of metam-sodium	MITC rate assuming complete conversion from metam-sodium* (kg/ha)	FIR/bw	RUD	ETE	TER	Annex VI Trigger value
strawberry	346 (612)	1.1	0.021	7.99	22.9	10
tomato	346 (612)	1.1	0.021	7.99	22.9	10
carrot	230 (408)	1.1	0.021	5.31	34.5	10
corn salad	173 (306)	1.1	0.021	4.00	45.9	10
potato	86.3 (153)	1.1	0.021	1.99	92.0	10

* metam-sodium application rate in brackets

Based on first tier assumptions (PD, PT = 1) and arithmetic mean measured residues, the short-term TER values for all field uses meet the trigger value of 10 for vermivorous birds.

In conclusion, the risk of metam-sodium and its metabolite MITC to birds is acceptable for the uses in greenhouse and localised use in vines. The risk is acceptable for the intended field uses in potato, corn salad and carrot (application rates 153, 306 and 408 kg a.s./ha). The risk is acceptable for the intended field use in strawberry (612 kg a.s./ha) due to the plastic film preventing exposure to birds. However, risk mitigation measures are needed for the intended field use in tomato (612 kg a.s./ha) since the acute TER value is below the trigger value and the fresh soil layer is not regarded sufficient to reduce the exposure to birds.

B.9.2 Effects on aquatic organisms (fish, aquatic invertebrates, algae) (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.1 Acute toxicity of the active substance and metabolites, degradation or reaction products to fish (Annex IIA 8.2.1)

Report on the Study of Acute Toxicity of Metam-Sodium in Rainbow Trout (*Salmo gairdneri* Rich.). (Gelbke H.P., Munk R., 1986a).

Guidelines :

EPA Guideline 72-1 : Freshwater fish LC₅₀

GLP :

No, study was performed prior to GLP implementation

Material and Methods :

Test substance : metam-sodium, 42.2 % aqueous solution, batch n° : ZH 130 585

Test species : *Oncorhynchus mykiss* (rainbow trout)

Number of organisms, weight, length : 10 fish per treatment, mean weight of 6.6 g (2.1 – 17.4 g), mean length of 8.3 cm (6.0 – 11.3 cm)

Type of test : 96 hours static toxicity test

Biological loading : 0.3 – 1.1 g fish/L

Applied and measured concentrations :

nominal : main study : control; 0.215, 0.464, 1.00, 2.15, 4.64 mg a.s./L

nominal : additional study : control; 1.00, 2.15, 4.64, 10.0, 21.5, 46.4, 100 mg a.s./L

measured after 1 hour : main study : control; 0.18, 0.40, 0.90, 1.90, 3.90 mg a.s./L

measured after 1 hour : additional study : control; 0.81, 2.01, 4.46, 9.07, 20.8, 44.2, 85.9 mg a.s./L

measured after 96 hours : main study : control; 0.08, -, -, - 0.80 mg a.s./L

measured after 96 hours : additional study : control; 0.62, 3.74, -, 20.1, 39.9, 87.8 mg a.s./L

Test conditions :

temperature : 11 - 14 °C

pH : 7.5 – 8.4

dissolved oxygen : 72 - 108 % O₂ saturation (7.7 – 11.6 mg/L O₂)

total hardness : 2.5 mmol/L

photoperiod : 16/8 hours light/dark cycle

Analytical methods : HPLC

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 0.215, 0.464, 1.00, 2.15, 4.64, 10.0 and 21.5 mg a.s./L after 96 hours of exposure. Mortality at the treatment levels of 46.4 and 100 mg a.s./L after 96 hours of exposure was 70 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment levels of 0.215 and 0.464 mg a.s./L. Clinical signs of toxicity observed from the treatment level of 1.00 mg a.s./L onwards were tumbling, discolouration, lying on the bottom, gasping and narcotic-like state.

Conclusions :

The study is not acceptable due to doubtful results. During the range finding test the LC₅₀ (*Oncorhynchus mykiss*, 96 h) was estimated between 0.316 and 1.00 mg a.s./L.

Endpoints :

LC₅₀ (*Oncorhynchus mykiss*, 96 h) = 40.28 mg metam-sodium/L (nominal)

LC₅₀ (*Oncorhynchus mykiss*, 96 h) = 35.18 mg metam-sodium/L (measured at 96 hours)

Potassium N-methyldithiocarbamate (PNMDC): Acute toxicity to Rainbow trout, *Oncorhynchus mykiss*, under flow-through test conditions. (Carr K.A., Wheat J.V., 1992).

Guidelines :

EPA Guideline 72-1 : Freshwater fish LC₅₀

GLP :

Yes

Material and Methods :

Test substance : metam-potassium (PNMDC), chemical purity : 54.0 %, batch n° : 1A-1275

Test species : *Oncorhynchus mykiss* (rainbow trout)

Number of organisms, weight, length : 20 fish per treatment, mean wet weight : 0.36 g (0.17 – 0.69 g),
mean length : 37 mm (29 – 45 mm)

Type of test : 96 hours flow-through toxicity test (15 L of test solution with 7.6 volume renewals per day)

Biological loading : 0.06 g fish/L/day

Applied and measured concentrations :

nominal : control; 13.5, 22.5, 37.4, 62.4, 104 mg a.s./L

mean measured : control; 13.3, 21.7, 37.0, 63.3, 106 mg a.s./L

mean measured : 96 – 102 % of nominal concentrations

Test conditions :

temperature : 11.6 – 15.4 °C

pH : 8.2 – 8.5

dissolved oxygen : 75 - 94 % O₂ saturation (8.1 – 10.2 mg/L O₂)

total hardness : 152 – 168 mg/L CaCO₃

photoperiod : 16/8 hours light/dark cycle

light intensity : 298 – 383 lux

Analytical methods : HPLC with UV-VIS detection

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 13.3 and 21.7 mg a.s./L after 96 hours of exposure. Mortality at the treatment levels of 37.0, 63.0 and 106 mg a.s./L after 96 hours of exposure was 5 %, 45 % and 100 % respectively.

Behavioural observations : Clinical signs of toxicity observed from the treatment level of 13.3 mg a.s./L onwards were discolouration, complete loss of equilibrium, fish lying on the bottom and immobility.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Oncorhynchus mykiss*, 96 h) = 62.4 mg metam-potassium/L (mean measured)

Vapam : Safety evaluation on Rainbow trout. (Howard D.J., Johnston C.D., 1971).

Guidelines :

No reference to guideline is given, however, similar to EPA Guideline 72-1 : Freshwater fish LC₅₀

GLP :

No, study was performed prior to GLP implementation

Material and Methods :

Test substance : Vapam, formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients,
batch n° : not reported

Test species : *Oncorhynchus mykiss* (rainbow trout)

Number of organisms, weight, length : 20 fish per treatment, mean weight of 0.73 g, mean length of 4.3 cm

Type of test : 96 hours static toxicity test

Biological loading : 0.24 g fish/L

Applied and measured concentrations :

nominal : control; 0.10, 0.18, 0.32, 0.56, 1.0 mg Vapam/L

corresponding to : control; 0.033, 0.059, 0.105, 0.183, 0.327 mg a.s./L

measured : not reported

Test conditions :

temperature : 11 – 12 °C

pH : not reported

dissolved oxygen : not reported

total hardness : not reported

photoperiod : not reported

Analytical methods : not reported

Findings :

Mortality : No mortalities occurred in the control group or at the treatment level of 0.10 mg Vapam/L after 96 hours of exposure. Mortality at the treatment levels of 0.18, 0.32, 0.56 and 1.0 mg Vapam/L after 96 hours of exposure was 5 %, 95 %, 100 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment levels of 0.10 and 0.18 mg Vapam/L. Clinical signs of toxicity observed at the treatment levels of 0.32, 0.56 and 1.0 mg Vapam/L were fish swimming near the surface, accompanied by a slight loss of equilibrium and side-swimming.

Conclusions :

The study is acceptable. The study shows a lot of shortcomings but gives a good indication of the toxicity level of metam-sodium.

Endpoints :

LC₅₀ (*Oncorhynchus mykiss*, 96 h) = 0.24 mg Vapam/L = 0.078 mg metam-sodium/L (nominal)

Methylisothiocyanate (metabolite of BAS 002 N, dazomet) acute toxicity study on the rainbow trout (*Oncorhynchus mykiss*) in a semistatic system over 96 hours. (Zok S., 2002).

Guidelines :

EPA Guideline 72-1 : Freshwater fish LC₅₀

EPA-SEP 540/9-85-006

EEC directive 92/69, Annex V, C1

OECD Guideline 203 : Fish Acute Toxicity Test

GLP :

Yes

Material and Methods :

Test substance : MITC, chemical purity : 99.6 %, batch n° : 408208/1 61100

Test species : *Oncorhynchus mykiss* (rainbow trout)

Number of organisms, age, weight, length : 7 fish per treatment, approximately 3 months old, mean weight of 1.0 g (0.8 – 1.2 g), mean length of 4.9 cm (4.8 – 5.1 cm)

Type of test : 96 hours semi-static toxicity test (medium renewal every 24 hours)

Biological loading : 0.64 g fish/L

Applied and measured concentrations :

nominal : control; 0.05, 0.1, 0.22, 0.5, 1.0 mg MITC/L

measured at start of the interval : 64.0 – 93.4 % of nominal concentrations

measured at end of the interval : 36.9 – 86.1 % of nominal concentrations

mean measured : 63.9 – 89.8 % of nominal concentrations

Test conditions :

temperature : 14 ± 1 °C

pH : 8.1 – 8.4

dissolved oxygen : 65- 103 % O₂ saturation (6.8 – 10.7 mg/L O₂)

total hardness : 250 mg/L CaCO₃

photoperiod : 16/8 hours light/dark cycle

Analytical methods : HPLC with UV-detection

Findings :

Mortality : No mortalities occurred in the control group or at the treatment level of 0.05 mg MITC/L after 96 hours of exposure. Mortality at the treatment levels of 0.1, 0.22, 0.5 and 1.0 mg MITC/L after 96 hours of exposure was 86 %, 100 %, 100 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment level of 0.1 mg MITC/L. At the treatment level of 0.1 mg MITC/L the only surviving fish was swimming at the bottom.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Oncorhynchus mykiss*, 96 h) = 0.08 mg MITC/L (nominal)

LC₅₀ (*Oncorhynchus mykiss*, 96 h) = 0.0531 mg MITC/L (mean measured)

NOEC (*Oncorhynchus mykiss*, 96 h) = 0.05 mg MITC/L (nominal)

NOEC (*Oncorhynchus mykiss*, 96 h) = 0.0320 mg MITC/L (mean measured)

The acute toxicity of MITC Technical to Rainbow trout, *Oncorhynchus mykiss*, in a flow through system (Schupner J., Stachura B., 1991a).

Guidelines :

EPA Guideline 72-1 : Freshwater fish LC₅₀

GLP :

No

Material and Methods :

Test substance : MITC, chemical purity : 94.9 %, batch n° : not reported

Test species : *Oncorhynchus mykiss* (rainbow trout)

Number of organisms, weight, length : 10 fish per replicate, 2 replicates per treatment, mean weight of 0.64 g, mean length of 34 mm

Type of test : 96 hours flow-through toxicity test (10 L of test solution with 8.6 volume renewals per day)

Biological loading : 0.074 g fish/L/day

Applied and measured concentrations :

nominal : control; solvent control (acetone); 32, 54, 90, 150, 250 µg MITC/L

mean measured : control; solvent control (acetone); 26, 40, 78, 131, 210 µg MITC/L

measured : 74 - 87 % of nominal concentrations

Test conditions :

temperature : 11.3 – 12.6 °C

pH : 6.2 – 6.5

dissolved oxygen : > 76 % O₂ saturation

total hardness : 44 mg/L CaCO₃

photoperiod : 16/8 hours light/dark cycle

Analytical methods : GC

Findings :

Mortality : No mortalities occurred in the control group, in the solvent control group or at the treatment levels of 26 and 40 µg MITC/L after 96 hours of exposure. Mortality at the treatment levels of 78, 131 and 210 µg MITC/L after 96 hours of exposure was 15 %, 100 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment levels of 26 and 40 µg MITC/L. Clinical signs of toxicity observed at the three highest treatment levels of 78, 131 and 210 µg MITC/L were lethargy, discoloration, reduced/labored breathing, reduced swimming, loss of equilibrium, spinal curvature and peritoneal lumps.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Oncorhynchus mykiss*, 96 h) = 0.094 mg MITC/L (mean measured)

NOEC (*Oncorhynchus mykiss*, 96 h) = 0.040 mg MITC/L (mean measured)

Report on the Study of Acute Toxicity of Metam-sodium in the Bluegill (*Lepomis Macrochirus Raf.*). (Gelbke H. P., Munk R., 1986b).

Guidelines :

EPA Guideline 72-1 : Freshwater fish LC₅₀

GLP :

No, study was performed prior to GLP implementation

Material and Methods :

Test substance : metam-sodium, 42.2 % aqueous solution, batch n° : ZH 130 585

Test species : *Lepomis macrochirus* (bluegill sunfish)

Number of organisms, weight, length : 10 fish per treatment, mean weight of 0.4 g, mean length of 3.3 cm (2.9 – 3.8 cm)

Type of test : 96 hours static toxicity test

Biological loading : 0.04 g fish/L

Applied and measured concentrations :

nominal : control; 0.100, 0.215, 0.464, 1.00, 2.15, 4.64, 10.0, 21.5 mg a.s./L

measured after 1 hour : control; 0.052, 0.194, 0.40, 0.75, 1.76, 4.36, 9.76, 18.95 mg a.s./L

measured after 96 hours : control; 0.07, 0.175, 0.095, 0.82, -, -, -, - mg a.s./L

Test conditions :

temperature : 22 – 23 °C

pH : 7.5 – 8.1

dissolved oxygen : 69 – 101 % O₂ saturation (6.0 – 8.8 mg/L O₂)

total hardness : 2.5 mmol/L

photoperiod : 16/8 hours light/dark cycle

Analytical methods : HPLC

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 0.100 and 0.215 mg a.s./L after 96 hours of exposure. Mortality at the treatment levels of 0.464, 1.00, 2.15, 4.64, 10.0 and 21.5 mg a.s./L after 96 hours of exposure was 10 %, 100 %, 100 %, 100 %, 100 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment levels of 0.100 and 0.215 mg a.s./L. At the treatment level of 21.5 mg a.s./L, clinical signs of toxicity observed after 48 hours of exposure were apathy and abnormal swimming behaviour.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Lepomis macrochirus*, 96 h) > 0.464 mg metam-sodium/L (nominal)

LC₅₀ (*Lepomis macrochirus*, 96 h) > 0.175 mg metam-sodium/L (measured at 96 hours)

Potassium N-methyldithiocarbamate (PNMDC): Acute toxicity to Bluegill, *Lepomis macrochirus*, under flow-through test conditions. (Lintott D.R., Wheat J.V., 1992).

Guidelines :

EPA Guideline 72-1 : Freshwater fish LC₅₀

GLP :

Yes

Material and Methods :

Test substance : metam-potassium (PNMDC), chemical purity : 54.0 %, batch n° : 1A-1275

Test species : *Lepomis macrochirus* (bluegill sunfish)

Number of organisms, age, weight, length : 20 fish per treatment, mean wet weight : 0.77 g (0.37 – 1.17 g), mean length : 30 mm (24 – 35 mm)

Type of test : 96 hours flow-through toxicity test (15 L of test solution with 7.4 volume renewals per day)

Biological loading : 0.14 g fish/L/day

Applied and measured concentrations :

nominal : control; 15.6, 26.0, 43.2, 72.0, 120 mg a.s./L

mean measured : control; 16.7, 26.7, 45.9, 76.5, 125 mg a.s./L

mean measured : 103 - 107 % of nominal concentrations

Test conditions :

temperature : 20.4 – 21.7 °C

pH : 8.1 – 8.4

dissolved oxygen : 80 – 93 % O₂ saturation (7.2 – 8.4 mg/L O₂)

total hardness : 124 mg/L CaCO₃

photoperiod : 16/8 hours light/dark cycle

light intensity : 318 – 511 lux

Analytical methods : HPLC with UV-VIS detection

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 16.7, 26.7 and 45.9 mg a.s./L after 96 hours of exposure. Mortality at the treatment levels of 76.5 and 125 mg a.s./L after 96 hours of exposure was 10 % and 70 % respectively.

Behavioural observations : Clinical signs of toxicity observed from the treatment level of 76.5 mg a.s./L onwards were dark pigmentation in the head region and on the entire fish.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Lepomis macrochirus*, 96 h) = 108 mg metam-potassium/L (mean measured)

Vapam Safety evaluation on Bluegill Sunfish. (Howard D.J., Johnston C.D., 1970a).

Guidelines :

No reference to guideline is given, however, similar to EPA Guideline 72-1 : Freshwater fish LC₅₀

GLP :

No, study was performed prior to GLP implementation

Material and Methods :

Test substance : Vapam, formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients, batch n° : not reported

Test species : *Lepomis macrochirus* (bluegill sunfish)

Number of organisms, weight, length : 5 fish per replicate, 4 replicates for the control and for the treatment levels of 1.0, 1.4 and 1.8 mg Vapam/L, 2 replicates for the treatment levels of 0.56, 3.2 and 10.0 mg Vapam/L, mean weight of 2.52 g, mean length of 5.1 cm

Type of test : 96 hours static toxicity test

Biological loading : 0.84 g fish/L

Applied and measured concentrations :

nominal : control; 0.56, 1.0, 1.4, 1.8, 3.2, 10.0 mg Vapam/L

corresponding to : control; 0.18, 0.33, 0.46, 0.59, 1.05, 3.27 mg a.s/L

measured : not reported

Test conditions :

temperature : 16.7 – 17.8 °C

pH : not reported

dissolved oxygen : not reported

total hardness : not reported

photoperiod : not reported

light intensity : not reported

Analytical methods : not reported

Findings :

Mortality : No mortalities occurred in the control group or at the treatment level of 0.56 mg Vapam/L after 96 hours of exposure. Mortality at the treatment levels of 1.0, 1.4, 1.8, 3.2 and 10.0 mg Vapam/L after 96 hours of exposure was 20 %, 75 %, 100 %, 100 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment level of 0.56 mg Vapam/L. Clinical signs of toxicity observed at the treatment levels of 1.0, 1.4, 1.8, 3.2 and 10.0 mg Vapam/L were fish swimming near the surface accompanied by a slight loss of equilibrium and side swimming. The most severe signs were seen at the three highest treatment levels.

Conclusions :

The study is acceptable. The study shows a lot of shortcomings but gives a good indication of the toxicity level of metam-sodium.

Endpoints :

LC₅₀ (*Lepomis macrochirus*, 96 h) = 1.19 mg Vapam/L = 0.389 mg metam-sodium/L (nominal)

The acute toxicity of MITC Technical to Bluegill Sunfish, *Lepomis macrochirus*, in a flow through system (Schupner J., Stachura B., 1991b).

Guidelines :

EPA Guideline 72-1 : Freshwater fish LC₅₀

GLP :

No

Material and Methods :

Test substance : MITC, chemical purity : 94.9 %, batch n° : not reported

Test species : *Lepomis macrochirus* (bluegill sunfish)

Number of organisms, weight, length : 10 fish per replicate, 2 replicates per treatment, mean weight of 0.608 g, mean length of 29 mm

Type of test : 96 hours flow-through toxicity test (10 L of test solution with 8.6 volume renewals per day)

Biological loading : 0.071 g fish/L/day

Applied and measured concentrations :

nominal : control; solvent control (acetone); 64.8, 108, 180, 300, 500 µg MITC/L

mean measured : control; solvent control (acetone); 48, 88, 157, 251, 398 µg MITC/L

measured : 74- 87 % of nominal concentrations

Test conditions :

temperature : 21.1 – 22.9 °C

pH : 6.3 – 7.1

dissolved oxygen : > 83 % O₂ saturation

total hardness : 42 mg/L CaCO₃

photoperiod : 16/8 hours light/dark cycle

Analytical methods : not reported

Findings :

Mortality : No mortalities occurred in the control group, in the solvent control group or at the treatment levels of 48 and 88 µg MITC/L after 96 hours of exposure. Mortality at the treatment levels of 157, 251 and 398 µg MITC/L after 96 hours of exposure was 65 %, 100 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment levels of 48 and 88 µg MITC/L. Clinical signs of toxicity observed at the three highest treatment levels of 157, 251 and 398 µg MITC/L were sounding, dark pigmentation and lethargy.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Lepomis macrochirus*, 96 h) = 0.142 mg MITC/L (mean measured)

NOEC (*Lepomis macrochirus*, 96 h) = 0.088 mg MITC/L (mean measured)

Potassium N-methyldithiocarbamate (PNMDC): Acute toxicity to the Sheepshead Minnow, *Cyprinodon variegatus*, under flow-through test conditions. (Sutherland G.D., Lintott D.R., 1992).

Guidelines :

EPA Guideline 72-3 : Acute LC₅₀ estuarine and marine organisms

GLP :

Yes

Material and Methods :

Test substance : metam-potassium (PNMDC), chemical purity : 54.0 %, batch n° : 1A-1275

Test species : *Cyprinodon variegatus* (sheepshead minnow)

Number of organisms, weight, length : 20 fish per treatment, mean wet weight : 1.59 g (0.98 – 2.63 g), mean length : 33 mm (27 – 37 mm)

Type of test : 96 hours flow-through toxicity test (42 L of test solution with 6.2 volume renewals per day)

Biological loading : 0.12 g fish/L/day

Applied and measured concentrations :

nominal : control; 7.8, 13, 22, 36, 60 mg a.s./L

mean measured : control; 6.4, 10, 22, 38, 66 mg a.s./L

mean measured : 77 – 110 % of nominal concentrations

Test conditions :

temperature : 21.7 – 23.8 °C

pH : 8.0 – 8.4

dissolved oxygen : > 79 % O₂ saturation (> 6.0 mg/L O₂)

salinity : 16 – 18 ‰

photoperiod : 16/8 hours light/dark cycle

Analytical methods : HPLC with UV-VIS detection

Findings :

Mortality : No mortalities occurred in the control groups or at the treatment levels of 6.4, 10 and 22 mg a.s./L after 96 hours of exposure. Mortality at the treatment levels of 38 and 66 mg a.s./L after 96 hours of exposure was 95 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment level of 6.4 mg a.s./L. At the treatment level of 10 mg a.s./L two fish were continuously at the water surface. At the treatment level of 22 mg a.s./L two fish showed complete loss of equilibrium and 18 fish were at the water surface. The only surviving fish at the treatment level of 38 mg a.s./L was lying on its side on the bottom. At the highest treatment level of 66 mg a.s./L all fish were dark after 24 hours of exposure.

Conclusions :

The study is acceptable.

Endpoints :

LC₅₀ (*Cyprinodon variegatus*, 96 h) = 30 mg metam-potassium/L (mean measured)

NOEC (*Cyprinodon variegatus*, 96 h) = 6.4 mg metam-potassium/L (mean measured)

Vapam - Safety evaluation on *Cyprinodon variegatus* (Broad Killifish). (Howard D.J., Johnston C.D., 1970b).

Guidelines :

No reference to guideline is given, however, similar to EPA Guideline 72-3 : Acute LC₅₀ estuarine and marine organisms

GLP :

No, study was performed prior to GLP implementation

Material and Methods :

Test substance : Vapam, formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients, batch n° : not reported

Test species : *Cyprinodon variegatus* (sheepshead minnow)

Number of organisms, weight, length : 5 fish per replicate, 4 replicates for the control and for the treatment levels of 1.8 and 3.2 mg Vapam/L, 2 replicates for the treatment levels of 0.56, 1.0, 5.6 and 10.0 mg Vapam/L, mean weight of 1.29 g, mean length of 3.7 cm

Type of test : 96 hours static toxicity test

Biological loading : 0.43 g fish/L

Applied and measured concentrations :

nominal : control; 0.56, 1.0, 1.8, 3.2, 5.6, 10.0 mg Vapam/L

corresponding to : control; 0.18, 0.33, 0.59, 1.05, 1.83, 3.27 mg a.s./L

measured : not reported

Test conditions :

temperature : 15 – 17.8 °C

pH : not reported

dissolved oxygen : not reported

salinity : not reported

photoperiod : not reported

Analytical methods : not reported

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 0.56 and 1.0 mg Vapam/L after 96 hours of exposure. Mortality was 100 % at the treatment levels of 1.8, 3.2, 5.6 and 10.0 mg Vapam/L after 96 hours of exposure.

Behavioural observations : No clinical signs of toxicity were observed at the treatment levels of 0.56 and 1.0 mg Vapam/L. Clinical signs of toxicity observed at the treatment levels of 1.8, 3.2 and 5.6 mg Vapam/L were fish swimming near the surface accompanied by a slight loss of equilibrium and side-swimming. At the highest treatment level of 10.0 mg Vapam/L, death ensued almost immediately after introduction of the test material and without any other clinical signs.

Conclusions :

The study is acceptable. The study shows a lot of shortcomings but gives a good indication of the toxicity level of metam-sodium.

Endpoints :

LC₅₀ (*Cyprinodon variegatus*, 96 h) = 1.3 mg Vapam/L = 0.425 mg metam-sodium/L (nominal)

Vapam Safety evaluation on Striped *Majatis*. (Howard D.J., Johnston C.D., 1970c).

Guidelines :

No reference to guideline is given, however, similar to EPA Guideline 72-3 : Acute LC₅₀ estuarine and marine organisms

GLP :

No, study was performed prior to GLP implementation

Material and Methods :

Test substance : Vapam, formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients, batch n° : not reported

Test species : striped *Majatis*

Number of organisms, weight, length : 5 fish per replicate, 4 replicates for the control and for the treatment levels of 1.8, 3.2 and 5.6 mg Vapam/L, 2 replicates for the treatment levels of 0.56, 1.0 and 10.0 mg Vapam/L, mean weight of 1.07 g, mean length of 4.4 cm

Type of test : 96 hours static toxicity test

Biological loading : 0.36 g fish/L

Applied and measured concentrations :

nominal : control; 0.56, 1.0, 1.8, 3.2, 5.6, 10.0 mg Vapam/L

corresponding to : control; 0.18, 0.33, 0.59, 1.05, 1.83, 3.27 mg a.s./L

measured : not reported

Test conditions :

temperature : 16.7 – 18.9 °C

pH : not reported

dissolved oxygen : not reported

total hardness : not reported

photoperiod : not reported

Analytical methods : not reported

Findings :

Mortality : No mortalities occurred in the control group or at the treatment levels of 0.56 and 1.0 mg Vapam/L after 96 hours of exposure. Mortality at the treatment levels of 1.8, 3.2, 5.6 and 10.0 mg Vapam/L after 96 hours of exposure was 85 %, 100 %, 100 % and 100 % respectively.

Behavioural observations : No clinical signs of toxicity were observed at the treatment levels of 0.56 and 1.0 mg Vapam/L. Clinical signs of toxicity observed at the treatment levels of 1.8, 3.2 and 5.6 mg Vapam/L were fish swimming near the surface accompanied by a slight loss of equilibrium and side-swimming. At the highest treatment level of 10.0 mg Vapam/L, death ensued almost immediately after introduction of the test material and without any other clinical signs.

Conclusions :

The study is acceptable. The study shows a lot of shortcomings but gives a good indication of the toxicity level of metam-sodium.

Endpoints :

LC₅₀ (striped *Majatis*, 96 h) = 2.5 mg Vapam/L = 0.491 mg metam-sodium/L (nominal)

B.9.2.2 Fish juvenile growth test (Annex IIA 8.2.2.1)

Due to a pronounced instability of metam-sodium in water and sediment (DT_{50} of metam-sodium (whole system) = 19.72 min and DT_{90} of metam-sodium (whole system) = 65.51 min), the predominant metabolite in water is MITC, rather than the parent metam-sodium. Therefore, MITC is considered as the only relevant residue for chronic aquatic risk assessment.

Sublethal toxic effects on rainbow trout (*Salmo gairdneri* RICH. = *Oncorhynchus mykiss*) of Methylisothiocyanate (MITC). (Munk R., 1990).

Guidelines :

OECD Guideline 204 : Fish, Prolonged Toxicity Test : 14-day study

German Federal Environmental Protection Agency : I 4.5-97125-2/6 : Test guideline "Sublethal toxic effects in the zebrafish *Brachidanio rerio*"

GLP :

Yes

Material and Methods :

Test substance : MITC, chemical purity : 98.4 %, batch n° : CP 4011

Test species : *Oncorhynchus mykiss* (rainbow trout)

Number of organisms, age, weight, length : 20 fish per treatment, approximately 7 months old, mean weight of 7.9 g (5.6 – 10.4 g), mean length of 8.9 cm (7.2 – 10.2 cm)

Type of test : 28-day flow-through toxicity test (7.2 volume replacements per day)

Biological loading : 0.22 g fish/L/day

Applied and measured concentrations :

nominal : control; solvent control (acetone); 0.001, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2 mg MITC/L

measured : 57 – 107 % of nominal concentrations

Feeding : "Ssniff" trout starter, about 4 % of their mean body weight daily

Test conditions :

temperature : 15 °C

pH : 7.5 – 7.9

dissolved oxygen : 75 – 107 % O₂ saturation (7.7 – 10.9 mg/L O₂)

total hardness : 2.3 – 2.4 mmol/L

photoperiod : 16/8 hours light/dark cycle

Analytical methods : reversed phase HPLC with UV-detection

Findings :Table B.9.2.2-1 : Summary of effects of MITC during the fish juvenile growth test with *Oncorhynchus mykiss*

Evaluation criteria	Control	Solvent control	Nominal test concentration (mg MITC/L)						
			0.001	0.005	0.01	0.02	0.05	0.1	0.2
Cumulative mortality after 28 days (%)	0	0	0	0	0	25	60	100	100
Mean body weight at day 0 (g)	7.69	8.20	7.91	7.94	8.18	8.11	7.84	7.49	7.57
Mean body weight at day 28 (g)	19.94	21.50	21.18	17.86	14.39*	10.28*	9.10*	-	-
Mean body length at day 0 (cm)	8.93	9.17	9.02	9.09	9.10	9.09	9.01	8.58	8.53
Mean body length at day 28 (cm)	12.04	12.25	12.20	11.73	10.57*	9.41*	9.39*	-	-

* statistically significantly different from the control

Behavioural observations : No abnormalities were detected in the fish of the control, the solvent control or at the treatment levels of 0.001 and 0.005 mg MITC/L. Surviving fish at the treatment level of 0.01 mg MITC/L suffered from spasms, convulsions, were lying on the bottom and had discoloration (dark). Surviving fish at the treatment levels of 0.02 and 0.05 mg MITC/L showed apathy, were swimming near the bottom, suffered from spasms, convulsions, were lying on the bottom and had discoloration (dark).

Conclusions :

The study is acceptable.

Endpoints :NOEC (*Oncorhynchus mykiss*, 28 d) = 0.005 mg MITC/L (nominal)

Added in August 2008, according to PRAPeR 53:

NOEC (*Oncorhynchus mykiss*, 28 d) = 0.004 mg MITC/L (mean measured concentrations)

For the recalculation of the endpoint in mean measured concentrations, the mean recovery of the concentration 0.05 mg MITC/L was used, being 80 %.

The NOEC is based on the effects on growth (body weight and body length) and clinical signs.

B.9.2.3 Fish early life stage toxicity (Annex IIA 8.2.2.2)

B.9.2.4 Fish life cycle test (Annex IIA 8.2.2.3)

Due to a pronounced instability of metam-sodium in water and sediment (DT_{50} of metam-sodium (whole system) = 19.72 min and DT_{90} of metam-sodium (whole system) = 65.51 min), the predominant metabolite in water is MITC, rather than the parent metam-sodium. Therefore, MITC is considered as the only relevant residue for chronic aquatic risk assessment.

A fish early life stage toxicity test with MITC is not required since a fish juvenile growth test was submitted to address the chronic toxicity.

A fish life cycle toxicity test with MITC is not required since the DT_{90} of MITC in water is less than 100 days. (DT_{90} of MITC (whole system) = 527.37 min).

B.9.2.5 Bioaccumulation potential in fish (Annex IIA 8.2.3)

A bioaccumulation test with metam-sodium is not required since the $\log P_{OW}$ value for metam-sodium is - 2.91, which is far below the trigger value of 3.

A bioaccumulation test with the main metabolite MITC is also not required since the $\log P_{OW}$ value for MITC is 1.05, also lower than the trigger value of 3.

B.9.2.6 Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4)

Determination of the Acute Toxicity of BASF 005 00 N Metam-sodium 510 g/l to the Waterflea *Daphnia magna* Straus. (Bias Merz, 1985).

Guidelines :

EPA Guideline 72-2 : Acute LC₅₀ freshwater invertebrates

GLP :

No, study was performed prior to GLP implementation

Material and Methods :

Test substance : Metam Fluid 510 g/L (BAS 005 00 N), formulation containing 42.2 % metam-sodium, batch n° : not reported

Test species : *Daphnia magna* (waterflea)

Number of organisms, age : 5 daphnids per replicate, 4 replicates per treatment, 6 – 24 hours old

Type of test : 48 hours static toxicity test

Applied and measured concentrations :

nominal : control; 0.122, 0.244, 0.488, 0.976, 1.95, 3.9, 7.81, 15.6, 31.2, 62.5 mg Metam Fluid/L

measured : not reported

Test conditions :

temperature : 20 °C

pH : 7.99 – 8.89 (at 0 hours), 7.84 – 7.91 (at 48 hours)

dissolved oxygen : 90 – 93 % O₂ saturation (8.29 – 8.6 mg/L O₂)

total hardness : 2.68 mmol/L

photoperiod : no lighting, except for the determination of the ability to swim

Analytical methods : not reported

Findings :

Mortality : No immobility was observed in the control group and up to the treatment level of 0.976 mg Metam Fluid/L after 48 hours of exposure. Immobility at the treatment levels of 1.95, 3.9, 7.81, 15.6, 31.2 and 62.5 mg Metam Fluid/L after 48 hours of exposure was 55 %, 70 %, 100 %, 100 %, 100 % and 100 % respectively.

Conclusions :

The study is acceptable.

Endpoints :

EC₅₀ (*Daphnia magna*, 48 h) = 2.34 mg Metam Fluid/L = 0.99 mg metam-sodium/L (nominal)

NOEC (*Daphnia magna*, 48 h) = 0.976 mg Metam Fluid/L = 0.41 mg metam-sodium/L (nominal)

Potassium N-methyldithiocarbamate (PNMDC): Acute toxicity to the water flea, *Daphnia Magna*, under flow-through test conditions. (Ward G.S., 1993).

Guidelines :

EPA Guideline 72-2 : Acute LC₅₀ freshwater invertebrates

GLP :

Yes

Material and Methods :

Test substance : metam-potassium (PNMDC), chemical purity : 54.0 %, batch n° : 1A-1275

Test species : *Daphnia magna* (waterflea)

Number of organisms, age : 10 daphnids per replicate, 2 replicates per treatment, less than 24 hours old

Type of test : 48 hours flow-through toxicity test (19 volume renewals per day)

Applied and measured concentrations :

nominal : control; 1.4, 2.3, 3.9, 6.5, 10.8, 18.0 mg a.s./L

mean measured : control; 1.34, 2.44, 4.68, 6.63, 11.2, 19.1 mg a.s./L

mean measured : 96 - 120 % of nominal concentrations

Test conditions :

temperature : 20.0 – 20.3 °C

pH : 6.7 – 7.0

dissolved oxygen : > 77 % O₂ saturation (> 7.0 mg/L O₂)

total hardness : 56 mg/L CaCO₃

photoperiod : 16/8 hours light/dark cycle

light intensity : 282 – 310 lux

Analytical methods : HPLC with UV-VIS detection

Findings :

Mortality : No immobility was observed in the control group and up to the treatment level of 4.68 mg a.s./L after 48 hours of exposure. Immobility at the treatment levels of 6.63, 11.2 and 19.1 mg a.s./L after 48 hours of exposure was 60 %, 75 % and 80 % respectively.

Behavioural observations : Lethargy was observed in one or more daphnids after 48 hours of exposure at the treatment levels of 2.44 mg a.s./L and higher.

Conclusions :

The study is acceptable.

Endpoints :

EC₅₀ (*Daphnia magna*, 48 h) = 6.34 mg metam-potassium/L (mean measured)

NOEC (*Daphnia magna*, 48 h) = 1.34 mg metam-potassium/L (mean measured)

Effect of Methyl-iso-thiocyanate (MITC) on the Immobility of *Daphnia magna* STRAUS in a 48 Hour Semi-Static, Acute Toxicity Test. (Dohmen G.P., 2002).

Guidelines :

OECD Guideline 202 : *Daphnia* sp., Acute Immobilisation Test
EEC directive 79/831, Annex V, C 2 : Acute Toxicity for *Daphnia*
EPA Guideline 72-2 : Acute LC₅₀ freshwater invertebrates
OPPTS 850.1010

GLP :

Yes

Material and Methods :

Test substance : MITC, chemical purity : 99.6 %, batch n° : 408208/1

Test species : *Daphnia magna* (waterflea)

Number of organisms, age : 5 daphnids per replicate, 4 replicates per treatment, neonates, less than 24 hours old

Type of test : 48 hours semi-static toxicity test (medium renewal after 24 hours)

Applied and measured concentrations :

nominal : control; solvent control (acetone); 0.050, 0.089, 0.158, 0.281, 0.50 mg MITC/L

mean measured : control; solvent control (acetone); 0.026, 0.05, 0.115, 0.132, 0.304 mg MITC/L

measured at 1st medium renewal : 67 – 97.1 % of nominal (0 hours), 23 – 35.4 % of nominal (24 hours)

measured at 2nd medium renewal : 78.4 – 85.9 % of nominal (0 hours), 31.1 – 41.2 % of nominal (24 hours)

Test conditions :

temperature : 21.0 – 23.5 °C

pH : 8.04 – 8.24

dissolved oxygen : 94 - 101 % O₂ saturation (8.3 – 8.9 mg/L O₂)

total hardness : 2.43 – 2.47 mmol/L

photoperiod : 16/8 hours light/dark cycle

light intensity : < 1500 lux

Analytical methods : HPLC with UV-detection

Findings :

Mortality : No immobility was observed in the control group, in the solvent control group and up to the treatment level of 0.05 mg MITC/L after 48 hours of exposure. Immobility was 100 % at the treatment levels of 0.115, 0.132 and 0.304 mg MITC/L after 48 hours of exposure.

Conclusions :

The study is acceptable.

Endpoints :

EC₅₀ (*Daphnia magna*, 48 h) = 0.076 mg MITC/L (mean measured)

Toxicity Research with Thiourea. (Maas J.L., 1990).

No study report is available to verify the toxicity endpoints. Therefore, the study is not acceptable.

The acute toxicity of MITC to *Daphnia magna* in a flow through system. (Schupner J., 1991c).

Guidelines :

EPA Guideline 72-2 : Acute LC₅₀ freshwater invertebrates

GLP :

No

Material and Methods :

Test substance : MITC, chemical purity : 95.0 %, batch n° : not reported

Test species : *Daphnia magna* (waterflea)

Number of organisms, age : 20 daphnids per treatment, no information on replicates, less than 24 hours old

Type of test : 48 hours flow-through toxicity test (22 volume replacements per day)

Applied and measured concentrations :

nominal : control; solvent control (acetone); 8, 14, 24, 45, 64 µg MITC/L

measured : not reported

Test conditions :

temperature : 20.5 °C

pH : 7.3

dissolved oxygen : 100 % O₂ saturation (9.1 mg/L O₂)

total hardness : not reported

photoperiod : 16/8 hours light/dark cycle

Analytical methods : not reported

Findings :

Mortality : No results were reported.

Conclusions :

The study is not acceptable due to several shortcomings (no information on the preparation of the test solutions and the concentration of the solvent, no information on the recovery of the test substance, no raw data on mortality and behavioural observations, no statistical analysis of the raw data)

Potassium N-methyldithiocarbamate (PNMDC): Acute toxicity to the Mysid, *Mysidopsis bahia*, Under Flow Through Test Conditions. (Juarovisech K.S., Lintott D., 1992).

Guidelines :

EPA Guideline 72-3 : Acute LC₅₀ estuarine and marine organisms

GLP :

Yes

Material and Methods :

Test substance : metam-potassium (PNMDC), chemical purity : 54.0 %, batch n° : 1A-1275

Test species : *Mysidopsis bahia* (mysids)

Number of organisms, age : 10 mysids per replicate, 2 replicates per treatment,
post-larval mysids (less than 24 hours old)

Type of test : 96 hours flow-through toxicity test (7.0 volume replacements per day)

Applied and measured concentrations :

nominal : control; 1.3, 2.2, 3.6, 6.0, 10 mg a.s./L

mean measured : control; 1.23, 1.78, 3.34, 5.92, 8.93 mg a.s./L

measured : 81 - 99 % of nominal concentrations

Feeding : mysids were fed live brine shrimp nauplii daily during the test to reduce cannibalism

Test conditions :

temperature : 19.6 – 23.2 °C

pH : 8.2 – 8.4

dissolved oxygen : 81 – 85 % O₂ saturation (7.2 – 7.6 mg/L O₂)

salinity : 20 ‰

photoperiod : 16/8 hours light/dark cycle

light intensity : 367 – 667 lux

Analytical methods : HPLC with UV-VIS detection

Findings :

Mortality : No mortalities occurred in the control group or up to the treatment level of 1.23 mg a.s./L after 96 hours of exposure. Mortality at the treatment levels of 1.78, 3.34, 5.92 and 8.93 mg a.s./L after 96 hours of exposure was 45 %, 60 %, 55 % and 100 % respectively.

Conclusions :

The study is acceptable.

Endpoints :

EC₅₀ (*Mysidopsis bahia*, 96 h) = 2.19 mg metam-potassium/L (mean measured)

NOEC (*Mysidopsis bahia*, 96 h) = 1.23 mg metam-potassium/L (mean measured)

Potassium N-methyldithiocarbamate (PNMDC): Acute effect on the new shell growth of Eastern Oyster *Crassostrea virginica*. (Lintott D.R., Ward S.G., 1993).

Guidelines :

EPA Guideline 72-3 : Acute LC₅₀ estuarine and marine organisms

GLP :

Yes

Material and Methods :

Test substance : metam-potassium (PNMDC), chemical purity : 54.0 %, batch n° : 1A-1275

Test species : *Crassostrea virginica* (eastern oyster)

Oysters used for testing were from a population with an umbo to distal valve edge length of approximately 23 to 33 mm (mean length of 27.3 mm, standard deviation of 2.8 mm).

Upon receipt, approximately 2 to 5 mm of shell growth was removed from the edge of each oyster with a high speed grinder to create a clean flat surface from which to measure new shell growth. These oysters were then added to a control tank in the test system for 2 days prior to test initiation.

Prior to initiating the study, any new shell growth that was present on the oysters was removed.

Number of organisms, length : 20 oysters per replicate, 27.3 ± 2.8 mm

Type of test : 96 hours flow-through toxicity test (89 volume replacements per day)

Applied and measured concentrations :

nominal : control; 3.9, 6.5, 11, 18, 30, 50 mg a.s./L

mean measured : control; 3.22, 5.62, 9.54, 17.1, 28.1, 49.0 mg a.s./L

measured : 83 – 98 % of nominal concentrations

Feeding : oysters were fed the marine diatom *Skeletonema costatum* twice per day during the test in an effort to boost new shell growth

Test conditions :

temperature : 24.9 – 26.7 °C

pH : 8.0 – 8.2

dissolved oxygen : > 88 % O₂ saturation (> 6.0 mg/L O₂)

salinity : 30 – 34 ‰

photoperiod : 16/8 hours light/dark cycle

light intensity : 800 – 975 lux

Analytical methods : LC with UV-VIS detection

Findings :

Mortality : No mortalities occurred in the control group or in any of the treatment groups after 96 hours of exposure.

Table B.9.2.6-1 : New shell growth of the Eastern Oyster (*Crassostrea virginica*) exposed for 96 hours und flow-through conditions to metam-potassium

Evaluation criteria	Control	Mean measured concentration (mg a.s./L)					
		3.22	5.62	9.54	17.1	28.1	49.0
New shell growth (mm)	2.32	1.71	1.28	0.83	0.55	0.05	0.00
Difference from the control	-	-0.61	-1.04	-1.49	-1.77	-2.27	-2.32
Percent change	-	-26*	-45*	-64*	-76*	-98*	-100*

* statistically significantly different from the control

Conclusions :

The study is acceptable.

Endpoints :

EC₅₀ (*Crassostrea virginica*, 96 h) = 6.45 mg metam-potassium/L (mean measured) based on new shell growth

NOEC (*Crassostrea virginica*, 96 h) < 3.22 mg metam-potassium/L (mean measured) based on new shell growth

B.9.2.7 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

Due to a pronounced instability of metam-sodium in water and sediment (DT_{50} of metam-sodium (whole system) = 19.72 min and DT_{90} of metam-sodium (whole system) = 65.51 min), the predominant metabolite in water is MITC, rather than the parent metam-sodium. Therefore, MITC is considered as the only relevant residue for chronic aquatic risk assessment.

Methylisothiocyanat - Determination of the chronic effect on the reproduction of the water flea *Daphnia magna* STRAUS. (Jatzek, 2001).

Guidelines :

OECD Guideline 202 : Part II : *Daphnia* sp., Reproduction Test

GLP :

Yes

Material and Methods :

Test substance : MITC, chemical purity : not reported, batch n° : 340 401/1

Test species : *Daphnia magna*

Number of organisms, age : 1 daphnid per replicate, 10 replicates per treatment, 2 – 24 hours old

Type of test : 21-day semi-static toxicity test (medium renewal on Monday, Wednesday and Friday)

Applied and measured concentrations :

nominal : control; 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 µg MITC/L

measured : 80 – 96 % of nominal

Feeding : live green algae (*Desmodesmus subspicatus*) daily

Test conditions :

temperature : 20.3 – 20.6 °C

pH : 7.0 – 8.2

dissolved oxygen : 49 - 103 % O₂ saturation (4.5 – 9.5 mg/L O₂)

total hardness : 2.20 – 3.20 mmol/L

photoperiod : 16/8 hours light/dark cycle

light intensity : 2 – 7 µE/(m².s)

Analytical methods : capillary gas chromatography and sulfur selective detector (FPD)

Findings :Table B.9.2.7-1 : Summary of effects of MITC during the reproduction study with *Daphnia magna*

Evaluation criteria	Control	Nominal test concentration (µg MITC/L)							
		0.78	1.56	3.13	6.25	12.5	25	50	100
Mortality of the parent daphnids (%)	0	0	20	0	0	10	30	100	100
Mean number of living young per surviving parent animal after 21-day exposure period	74.9	82.2	71.1	65.2	81.5	52.7	45.4*	-	-
Mean number of dead young per surviving parent animal after 21-day exposure period	10.1	9.0	8.8	12.4	8.8	10.1	13.6	-	-
Mean number of aborted subitane eggs per surviving parent animal after 21-day exposure period	1.0	0.9	1.1	0.6	0.8	0.6	1.6	-	-

* statistically significantly different from the control

Conclusions :

The study is acceptable.

Endpoints :NOEC (*Daphnia magna*, 21 d) = 0.00625 mg MITC/L (nominal)

The notifier proposed a NOEC of 0.0125 mg MITC/L based on statistics. However, a high variation in the results was observed for the control and all treatment groups (high standard deviation and high coefficient of variation). The number of living young per surviving parent animal after the 21-day exposure period ranged from 47 to 106 in the control. The sum of all replicates of living young per surviving parent animal after 21-day exposure period was 749 in the control, 815 at the treatment level of 6.25 µg MITC/L and only 474 at the treatment level of 12.5 µg MITC/L. Although the chronic effects at 12.5 µg MITC/L were not statistically significantly different from the control, the RMS considered them biologically relevant. By precautionary principle, the NOEC was set at 6.25 µg MITC/L.

B.9.2.8 Effects on algal growth (Annex IIA 8.2.6)

Metam sodium 510 g/l: Algal growth inhibition assay. (Jenkins C.A., 2003).

Guidelines :

EC Directive 92/69/EEC, Part C3

OECD Guideline 201 : Alga, Growth Inhibition Test

JMAFF Environmental Test Guidelines

EPA Guideline 122-2 : Aquatic plant growth

OPPTS 850.5400

GLP :

Yes

Material and Methods :

Test substance : Metam-Sodium, formulation containing 510 g a.s./L, batch n° : 32 E 28/6

Test species : *Pseudokirchneriella subcapitata* (green alga)

Number of replicates, initial cell density : 6 replicates for the control group, 3 replicates per treatment, 1×10^4 cells/mL

Type of test : 96 hours static toxicity test

Applied and measured concentrations :

nominal : control; 0.25, 0.5, 1, 2, 4, 8 mg Metam Sodium formulation/L
(equivalent to : control; 0.128, 0.255, 0.51, 1.02, 2.04, 4.08 mg a.s./L)

measured after 0 hours : control; 0.0601, 0.152, 0.322, 0.689, 1.42, 2.96 mg a.s./L

measured after 0 hours : 53 - 88 % of nominal concentrations

measured after 24 hours : no test substance was detected in the samples taken from the test cultures except at the highest test level where the measured level was 4 % of its nominal value

measured after 96 hours : no metam sodium was found in any of the test levels

Test conditions :

temperature : 22.4 – 24.0 °C

pH : 7.66 – 8.13 (at start), 7.85 – 9.91 (at end)

light regime : continuous

light intensity : 4070 – 4270 lux

Analytical methods : HPLC with spectrophotometric detector

Findings :

No microscopic abnormalities of the cells were detected.

Subcultures (established in freshly-prepared culture medium) from test cultures containing Metam Sodium 510 g/L at 4 and 8 mg/L (nominal) had re-established growth after 6 days of incubation, indicating that at these levels, the test substance was algistatic (suppressing growth).

Conclusions :

The study is acceptable.

Endpoints :

$E_b C_{50}$ (*Pseudokirchneriella subcapitata*, 72 h) = 1.69 mg Metam Sodium/L = 0.713 mg a.s./L (nominal)

$E_b C_{50}$ (*Pseudokirchneriella subcapitata*, 72 h) = 0.556 mg a.s./L (initially measured)

$E_b C_{50}$ (*Pseudokirchneriella subcapitata*, 96 h) = 1.67 mg Metam Sodium/L = 0.704 mg a.s./L (nominal)

$E_b C_{50}$ (*Pseudokirchneriella subcapitata*, 96 h) = 0.548 mg a.s./L (initially measured)

$E_r C_{50}$ (*Pseudokirchneriella subcapitata*, 72 h) = 3.08 mg Metam Sodium/L = 1.30 mg a.s./L (nominal)

$E_r C_{50}$ (*Pseudokirchneriella subcapitata*, 72 h) = 1.08 mg a.s./L (initially measured)

$E_r C_{50}$ (*Pseudokirchneriella subcapitata*, 96 h) = 3.22 mg Metam Sodium/L = 1.36 mg a.s./L (nominal)

$E_r C_{50}$ (*Pseudokirchneriella subcapitata*, 96 h) = 1.13 mg a.s./L (initially measured)

NOEC (*Pseudokirchneriella subcapitata*, 96 h) = 1 mg Metam Sodium/L = 0.422 mg a.s./L (nominal)

NOEC (*Pseudokirchneriella subcapitata*, 96 h) = 0.322 mg a.s./L (initially measured)

Effect of Methyl Isothiocyanate on the growth of the green alga *Pseudokirchneriella subcapitata*. (Kubitza J. 1998).

Guidelines :

OECD Guideline 201 : Alga, Growth Inhibition Test

GLP :

Yes

Material and Methods :

Test substance : MITC, chemical purity : 99.0 %, batch n° : 80525

Test species : *Pseudokirchneriella subcapitata* (green alga)

Number of replicates, initial cell density : 5 replicates for the control, 10 replicates for the solvent control, 5 replicates per treatment, 1×10^4 cells/mL

Type of test : 72 hours static toxicity test

Applied and measured concentrations :

nominal : control; solvent control (acetone); 0.03, 0.06, 0.10, 0.19, 0.35, 0.65, 1.2 mg MITC/L

initially measured : control; solvent control (acetone); 0.019, 0.041, 0.074, 0.134, 0.207, 0.384, 0.692 mg MITC/L

measured at test start : 57.7 – 74.2 % of nominal

measured at test end : 0 – 13.4 % of nominal

Test conditions :

temperature : 22 - 23 °C

pH : 8.0 at start, 7.82 – 8.03 at end

light regime : continuous

light intensity : 8000 lux

Analytical methods : HPLC with UV-detection

Findings :

The inhibition in biomass after 72 hours of exposure at the treatment levels of 0.019, 0.041, 0.074, 0.134, 0.207, 0.384, 0.692 mg MITC/L was 0.1 %, 0.0 %, 9.9 %, 10.8 %, 27.3 %, 71.9 % and 100.0 % respectively.

The inhibition in growth rate after 72 hours of exposure at the treatment levels of 0.019, 0.041, 0.074, 0.134, 0.207, 0.384, 0.692 mg MITC/L was 0.1 %, -0.1 %, 2.1 %, 2.0 %, 8.2 %, 34.1 % and 100.0 % respectively.

No morphological effects were observed.

Conclusions :

The study is acceptable.

Endpoints :

$E_b C_{50}$ (*Pseudokirchneriella subcapitata*, 72 h) = 0.28 mg MITC/L (initial measured)

$E_r C_{50}$ (*Pseudokirchneriella subcapitata*, 72 h) = 0.58 mg MITC/L (initial measured)

Effect of Methyl isothiocyanate (MITC) on the growth of the blue-green alga *Anabaena flos-aquae*. (Kubitza J. 2002).

Guidelines :

ASTM E 1218-90 : Conducting Static 96-h Toxicity Tests with Microalgae

OECD Guideline 201 : Alga, Growth Inhibition Test

OPPTS 850.1000

GLP :

Yes

Material and Methods :

Test substance : MITC, chemical purity : 99.6 %, batch n° : 408208/1

Test species : *Anabaena flos-aquae* (blue-green alga)

Number of replicates, initial cell density : 10 replicates for the control, 5 replicates per treatment, 3×10^4 cells/mL

Type of test : 72 hours static toxicity test

Applied and measured concentrations :

nominal : control; 1.0, 2.0, 3.8, 7.4, 14, 27, 50 mg MITC/L

initially measured : control; 0.9, 1.5, 2.7, 5.0, 8.9, 17.4, 28.9 mg MITC/L

measured at test start : 57.7 – 93.5 % of nominal

measured at test end : 3.5 – 5.7 % of nominal

Test conditions :

temperature : 24 ± 1 °C

pH : 7.5 at start, 7.44 – 7.63 at end

light regime : continuous

light intensity : 2300 lux

Analytical methods : HPLC with UV-detection

Findings and Conclusions :

The inhibition in biomass after 72 hours of exposure at the treatment levels of 0.9, 1.5, 2.7, 5.0, 8.9, 17.4, 28.9 mg MITC/L was -2.9 %, 48.9 %, 67.9 %, 87.7 %, 94.7 %, 96.3 % and 97.6 % respectively.

The inhibition in growth rate after 72 hours of exposure at the treatment levels of 0.9, 1.5, 2.7, 5.0, 8.9, 17.4, 28.9 mg MITC/L was -0.7 %, 22.3 %, 31.7 %, 70.6 %, 81.1 %, 94.1 % and 100.0 % respectively.

No morphological effects on the algae could be observed at the treatment levels up to 5.0 mg MITC/L. At 8.9 mg MITC/L a few, at 17.4 mg MITC/L a fourth and at the highest treatment level of 28.9 mg MITC/L all cells appeared deformed.

The study is acceptable.

Endpoints :

E_bC_{50} (*Anabaena flos-aquae*, 72 h) = 2.12 mg MITC/L (initial measured)

E_rC_{50} (*Anabaena flos-aquae*, 72 h) = 3.72 mg MITC/L (initial measured)

B.9.2.9 Effects on sediment dwelling organisms (Annex IIA 8.2.7)

Not required. Metam-sodium and its metabolite MITC do not accumulate or persist in aquatic sediments.

B.9.2.10 Effects on aquatic plants (Annex IIA 8.2.8)

Effect of methyl isothiocyanate (metabolite of BAS 002 N, Dazomet) on the growth of *Lemna gibba*. (Junker M., 2002).

Guidelines :

OECD Guideline 221 : *Lemna* sp., Growth Inhibition Test

ASTM E 1415-91 : Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3

EPA Guideline 123-2 : Aquatic plant growth

OPPTS 850.4400

GLP :

Yes

Material and Methods :

Test substance : MITC, chemical purity : 99.6 %, batch n° : 408208/1

Test species : *Lemna gibba* (duckweed)

Number of replicates, inoculum : 6 replicates for the control, 3 replicates per treatment, one plant with 4 fronds and two plants with 3 fronds, giving a total number of 10 fronds per replicate at test initiation

Type of test : 7-day semi-static toxicity test (medium renewal after 3 days)

Applied and measured concentrations :

nominal : control; 0.01, 0.031, 0.096, 0.298, 0.924, 2.863 mg MITC/L

mean measured : control; 0.010, 0.040, 0.090, 0.269, 0.942, 3.103 mg MITC/L

measured at 1st medium renewal : 79.1 – 115.5 % (0 hours), not detected after 3 days

measured at 2nd medium renewal : 100.5 – 177.8 % (0 hours), not detected after 4 days

The correct application of the lowest test concentration (below limit of quantitation) was verified by the determination of the stock solution of test beginning and renewal; recoveries of 102.1 % and 103.8 % were yielded.

The biological results are based on mean measured initial concentrations, except for the lowest concentration, which was based on nominal.

Test conditions :

temperature : 26 °C

pH : 7.53 – 7.59 (at start, day 0), 7.93 – 8.28 (aged solutions, day 3),

7.48 – 7.52 (after renewal, day 3), 8.72 – 9.01 (aged solutions, day 7)

light regime : continuous

light intensity : 7970 lux

Analytical methods : HPLC with UV-detection

Assessment : Frond production and appearance were recorded on days 3, 5 and 7.

Findings :

Table B.9.2.10-1 : Summary of effects of MITC during the growth inhibition test with *Lemna gibba*

Evaluation criteria	Control	Nom	Mean Measured Test Concentration (mg MITC/L)				
			0.010	0.040	0.090	0.269	0.942
Frond density after 7 days	155.33	158.00	143.33	139.33	129.67	52.67	12.67
Frond density inhibition (%)	-	-1.83	8.26	11.01	17.66*	70.64*	98.17*
Growth rate after 7 days	0.435	0.449	0.428	0.406	0.467	0.441	0.015
Growth rate inhibition (%)	-	-0.55	2.98	3.89	6.73*	39.38*	91.40*

* statistically significantly different from the control

Frond density = mean number of fronds per replicate after 7 days

Growth rate = mean growth rate after 7 days

The duckweed population in the control vessels grew sufficiently well, increasing from 10 fronds per vessel to an average of 155 fronds per vessel in the control after 7 days (corresponding to a 15.5 x multiplication or a doubling time of 1.8 days).

During the course of the study at a test concentration of 0.942 mg MITC/L, the fronds remained smaller and did not separate properly. Besides single fronds got brown and the plants showed shorter roots. At the highest test concentration of 3.103 mg MITC/L, plants lost their roots and got isolated fronds. Additionally, during the course of the study the fronds changed their colouring from brighter green to brown. At test end in the highest test concentration of 3.103 mg MITC/L, all fronds were white (dead). No unusual effects were observed at concentrations below 0.942 mg MITC/L.

Conclusions :

The study is acceptable.

Endpoints :

E_bC_{50} (*Lemna gibba*, 7 d) = 0.59 mg MITC/L (mean measured initial)

E_rC_{50} (*Lemna gibba*, 7 d) = 1.18 mg MITC/L (mean measured initial)

NOEC (*Lemna gibba*, 7 d) = 0.09 mg MITC/L (mean measured initial)

B.9.2.11 Acute toxicity of the preparations (Annex IIIA 10.2.1)

Not required. The studies with the formulations are reported under Annex II.

B.9.2.12 Microcosm and mesocosm study (Annex IIIA 10.2.2)

Not required. An acceptable risk of metam-sodium and its metabolite MITC has been proven for the intended uses with a no-spray zone of 20 m.

B.9.2.13 Residue data in fish (Annex IIIA 10.2.3)

Not required. Since the log P_{OW} of metam-sodium is - 2.91 and the log P_{OW} of MITC is 1.05, no residue data from fish are necessary, as no bioconcentration will occur.

B.9.2.14 Supplementary studies of toxicity to fish and aquatic invertebrates (Annex IIIA 10.2.4)

Not required.

B.9.2.15 Summary of effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

Table B.9.2.15-1 : Summary of effects of metam-sodium and metam-potassium on aquatic organisms

Test species	Test substance	Test system	Endpoints	References
<i>Oncorhynchus mykiss</i>	metam-potassium	96 h flow-through	LC ₅₀ = 62.4 mg a.s./L (mean measured)	Carr K.A., Wheat J.V., 1992
<i>Lepomis macrochirus</i>	metam-sodium	96 h static	LC ₅₀ > 0.175 mg a.s./L (measured at 96 hours)	Gelbke H.-P., Munk R., 1986b
<i>Lepomis macrochirus</i>	metam-potassium	96 h flow-through	LC ₅₀ = 108 mg a.s./L (mean measured)	Lintott D.R., Wheat J.V., 1992
<i>Cyprinodon variegatus</i>	metam-potassium	96 h flow-through	LC ₅₀ = 30 mg a.s./L (mean measured)	Sutherland G.D., Lintott D.R., 1992
<i>Daphnia magna</i>	metam-potassium	48 h flow-through	EC ₅₀ = 6.34 mg a.s./L (mean measured)	Ward G.S., 1993
<i>Mysidopsis bahia</i>	metam-potassium	96 h flow-through	EC ₅₀ = 2.19 mg a.s./L (mean measured)	Juarovisech K.S., Lintott D., 1992
<i>Crassostrea virginica</i>	metam-potassium	96 h flow-through	EC ₅₀ = 6.45 mg a.s./L (mean measured)	Lintott D.R., Ward S.G., 1993

Table B.9.2.15-2 : Summary of effects of the metabolite MITC on aquatic organisms

Test species	Test substance	Test system	Endpoints	References
<i>Oncorhynchus mykiss</i>	MITC	96 h semi-static	LC ₅₀ = 0.0531 mg MITC/L (mean measured)	Zok S., 2002
<i>Oncorhynchus mykiss</i>	MITC	96 h flow-through	LC ₅₀ = 0.094 mg MITC/L (mean measured)	Schupner J., Stachura B., 1991a
<i>Lepomis macrochirus</i>	MITC	96 h flow-through	LC ₅₀ = 0.142 mg MITC/L (mean measured)	Schupner J., Stachura B., 1991b
<i>Oncorhynchus mykiss</i>	MITC	28 d flow-through	NOEC = 0.004 mg MITC/L (mean measured)	Munk R., 1990
<i>Daphnia magna</i>	MITC	48 h semi-static	EC ₅₀ = 0.076 mg MITC/L (mean measured)	Dohmen G.P., 2002
<i>Daphnia magna</i>	MITC	21 d semi-static	NOEC = 0.00625 mg MITC/L (nominal)	Jatzek, 2001
<i>Pseudokirchneriella subcapitata</i>	MITC	72 h static	E _b C ₅₀ (72 h) = 0.28 mg MITC/L E _r C ₅₀ (72 h) = 0.58 mg MITC/L (initially measured)	Kubitza J., 1998
<i>Anabaena flos-aquae</i>	MITC	72 h static	E _b C ₅₀ (72 h) = 2.12 mg MITC/L E _r C ₅₀ (72 h) = 3.72 mg MITC/L (initially measured)	Kubitza J., 2002
<i>Lemna gibba</i>	MITC	7 d semi-static	E _b C ₅₀ (7 d) = 0.59 mg MITC/L E _r C ₅₀ (7 d) = 1.18 mg MITC/L (mean measured initial)	Junker M., 2002

Table B.9.2.15-3 : Summary of effects of the formulations Vapam, Metam Fluid 510 g/L and Metam Sodium 510 g/L on aquatic organisms

Test species	Test substance	Test system	Endpoints	References
<i>Oncorhynchus mykiss</i>	Vapam	96 h static	LC ₅₀ = 0.24 mg Vapam/L (0.078 mg metam-sodium/L) (nominal)	Howard D.J., Johnston C.D., 1971
<i>Lepomis macrochirus</i>	Vapam	96 h static	LC ₅₀ = 1.19 mg Vapam/L (0.389 mg metam-sodium/L) (nominal)	Howard D.J., Johnston C.D., 1970a
<i>Cyprinodon variegatus</i>	Vapam	96 h static	LC ₅₀ = 1.3 mg Vapam/L (0.425 mg metam-sodium/L) (nominal)	Howard D.J., Johnston C.D., 1970b
Striped <i>Majatis</i>	Vapam	96 h static	LC ₅₀ = 1.5 mg Vapam/L (0.491 mg metam-sodium/L) (nominal)	Howard D.J., Johnston C.D., 1970c
<i>Daphnia magna</i>	Metam Fluid 510 g/L	48 h static	EC ₅₀ = 2.34 mg Metam fluid/L (0.99 mg metam-sodium/L) (nominal)	Bias Merz, 1985
<i>Pseudokirchneriella subcapitata</i>	Metam-Sodium 510 g/L	96 h static	E _b C ₅₀ (72 h) = 0.556 mg a.s./L E _r C ₅₀ (72 h) = 1.08 mg a.s./L (initially measured)	Jenkins C.A., 2003

Vapam : formulation containing 32.7 % anhydrous metam-sodium and 67.3 % inert ingredients

Metam Fluid 510 g/L : formulation containing 42.2 % metam-sodium

Metam Sodium 510 g/L : formulation containing 510 g metam-sodium/L (batch n°: 32 E 28/6)

B.9.2.16 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)*Revised in August 2010:*

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil and the product is incorporated into the soil (soil injection or drip irrigation). Based on the application technique, drift of metam-sodium and its metabolite MITC to surface water can be excluded. Because of the rapid degradation of metam-sodium in soils (DT₅₀ between 4 and 17 minutes), surface water contamination with the parent molecule can be excluded. Aquatic organisms may be exposed to the metabolite MITC as a result of drainage or run-off from treated fields.

The indoor uses pose no concern for surface water contamination. (e.g. cucumber, eggplant/aubergine, pepper and tomato) whilst the outdoor uses on strawberries occur under a plastic film, thus also preventing connectivity between soil and run-off water. Therefore no MITC contamination of surface water bodies via run-off is possible for this use. The uses on grapes are as a spot treatment and are very localized. Hence the overall application rate (for instance over an entire hectare) will be much lower than the application rate stated by m².

The Predicted Environmental Concentrations of MITC in surface water were calculated for the use in carrot (1 x 408 kg a.s./ha), in corn salad (1 x 306 kg a.s./ha), in tomato (1 x 612 kg a.s./ha) and in potato (1 x 153 kg a.s./ha). Based on the high application rate of MITC and its high toxicity to aquatic organisms, step 1-2 calculations are not sufficiently demonstrating safe uses and are therefore not reported. The run-off/drainage contributions of 2-5 % of the application rate that are assumed in step 1-2 calculations would lead to unrealistically high PEC_{SW} levels.

Step 3-4 calculations, using PRZM, MACRO and TOXSWA in combination with the FOCUS surface water scenarios were conducted.

Summaries of initial PEC_{SW}, PEC TWAEC_{SW} 2 days, PEC TWAEC_{SW} 4 days, PEC TWAEC_{SW} 21 days and PEC TWAEC_{SW} 28 days for MITC in all waterbody scenarios at FOCUS Step 3 using laboratory soil degradation data are shown below, for carrots, corn salad, tomato and potato respectively.

Table B.9.2.16-1: Step 3 PEC_{SW} and PEC_{SED} for MITC following application to carrots (FOCUS-root vegetables)

Scenario	Waterbody	Max PEC _{SW} (µg/L)	Max PEC _{SED} (µg/kg)	1 day TWAEC _{SW} (µg/L)	2 day TWAEC _{SW} (µg/L)	4 day TWAEC _{SW} (µg/L)	7 day TWAEC _{SW} (µg/L)	14 day TWAEC _{SW} (µg/L)	21 day TWAEC _{SW} (µg/L)	28 day TWAEC _{SW} (µg/L)
D3	Ditch	0.2740	0.3760	0.2730	0.2730	0.2700	0.2670	0.2560	0.2450	0.2370
D6	Ditch	1.6640	0.1540	0.8840	0.6670	0.4540	0.3020	0.1730	0.1180	0.0891
R1	Pond	0.2840	0.0461	0.2190	0.1710	0.1110	0.0685	0.0411	0.0281	0.0211
R1	Stream	43.2370	2.4000	10.3130	5.1600	2.5810	1.4750	1.0580	0.7060	0.5290
R2	Stream	5.2130	0.5920	2.8640	1.8200	0.9360	0.5380	0.2700	0.1800	0.1350
R3	Stream	115.4920	7.9930	43.1230	21.5770	10.7920	6.1680	3.1000	2.0670	1.5500
R4	Stream	49.7660	5.8790	39.0220	21.1170	10.5870	6.0500	3.0250	2.0170	1.5130

Table B.9.2.16-2: Step 3 PEC_{SW} and PEC_{SED} for MITC following application to corn salad (FOCUS-leafy vegetables)

Scenario	Waterbody	Max PEC _{SW} (µg/L)	Max PEC _{SED} (µg/kg)	1 day TWAEC _{SW} (µg/L)	2 day TWAEC _{SW} (µg/L)	4 day TWAEC _{SW} (µg/L)	7 day TWAEC _{SW} (µg/L)	14 day TWAEC _{SW} (µg/L)	21 day TWAEC _{SW} (µg/L)	28 day TWAEC _{SW} (µg/L)
D3	Ditch	0.0002	0.0006	0.0002	0.0002	0.0001	0.0001	0.0001	0.0001	0.0001
D3	Ditch	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
D4	Pond	0.0129	0.0142	0.0128	0.0126	0.0120	0.0108	0.0100	0.0078	0.0063
D4	Stream	0.4280	0.3190	0.4240	0.4180	0.3980	0.3590	0.3230	0.2630	0.2180
D6	Ditch	0.0000	0.0000	N/A	N/A	N/A	N/A	N/A	N/A	N/A
R1	Pond	0.1230	0.0187	0.0935	0.0712	0.0447	0.0269	0.0168	0.0137	0.0103
R1	Stream	40.7640	3.2110	15.7520	7.8800	3.9410	2.2520	1.1850	0.7910	0.5930
R1	Pond	0.4290	0.0603	0.3270	0.2410	0.1420	0.0867	0.0452	0.0301	0.0226
R1	Stream	2.6630	0.3520	1.8580	0.9300	0.4650	0.3030	0.1530	0.1020	0.0767
R2	Stream	78.4160	6.5440	34.0310	17.0210	8.5120	4.8650	2.6250	1.7500	1.3120
R3	Stream	10.7750	1.4630	9.2480	4.7150	2.3590	1.3480	0.6740	0.4500	0.3370
R4	Stream	28.6220	3.2870	19.8350	9.9230	4.9630	3.0680	1.5400	1.0270	0.7700

N/A The simulated period was too short for calculation of PEC_{SW}Table B.9.2.16-3: Step 3 PEC_{SW} and PEC_{SED} for MITC following application to tomatoes (FOCUS-fruiting vegetables)

Scenario	Waterbody	Max PEC _{SW} (µg/L)	Max PEC _{SED} (µg/kg)	1 day TWAEC _{SW} (µg/L)	2 day TWAEC _{SW} (µg/L)	4 day TWAEC _{SW} (µg/L)	7 day TWAEC _{SW} (µg/L)	14 day TWAEC _{SW} (µg/L)	21 day TWAEC _{SW} (µg/L)	28 day TWAEC _{SW} (µg/L)
D6	Ditch	0.8410	0.4650	0.8210	0.7530	0.5820	0.4240	0.3360	0.3030	0.2900
R2	Stream	25.0930	2.7080	14.6930	8.4610	4.2740	2.4540	1.2270	0.8180	0.6140
R3	Stream	184.6650	15.8750	94.9340	47.5660	23.7960	13.6020	7.2900	4.8650	3.6490
R4	Stream	114.8120	6.5240	33.0880	17.8840	8.9440	5.1120	2.5860	1.7300	1.2970

Table B.9.2.16-4: Step 3 PEC_{SW} and PEC_{SED} for MITC following application to potatoes (FOCUS-potatoes)

Scenario	Waterbody	Max PEC _{SW} (µg/L)	Max PEC _{SED} (µg/kg)	1 day TWAEC _{SW} (µg/L)	2 day TWAEC _{SW} (µg/L)	4 day TWAEC _{SW} (µg/L)	7 day TWAEC _{SW} (µg/L)	14 day TWAEC _{SW} (µg/L)	21 day TWAEC _{SW} (µg/L)	28 day TWAEC _{SW} (µg/L)
D3	Ditch	0.0319	0.0728	0.0318	0.0318	0.0317	0.0316	0.0314	0.0309	0.0304
D4	Pond	0.5980	0.2530	0.5930	0.5780	0.5520	0.4950	0.3680	0.2940	0.2350
D4	Stream	3.3540	1.3000	2.8920	2.6940	2.6090	2.3530	1.7350	1.3910	1.1310
D6 (1 st crop)	Ditch	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
D6 (2 nd crop)	Ditch	9.0840	2.8030	8.3550	7.6220	6.8140	5.6700	3.7440	2.7030	2.1650
R1	Pond	0.0858	0.0126	0.0605	0.0436	0.0272	0.0181	0.0094	0.0063	0.0047
R1	Stream	13.0580	1.0790	4.3770	3.1090	1.5550	0.9120	0.4560	0.3040	0.2290
R2	Stream	0.1490	0.0170	0.0659	0.0444	0.0231	0.0133	0.0067	0.0044	0.0033
R3	Stream	0.3280	0.0397	0.1650	0.0904	0.0452	0.0262	0.0131	0.0087	0.0066

The PEC_{SW} values provided at Step 3 indicate that exposure concentrations from run-off into streams may need to be reduced in order to mitigate potential risks to aquatic organisms. Therefore run-off was reduced by 97.5%, consistent with buffer zones of 20 m (Step 4). The relevant PEC_{SW} values for simulations undertaken with laboratory soil data are shown below, for carrots, corn salad, tomato and potato respectively.

Table B.9.2.16-5: Step 4 PEC_{SW} and PEC_{SED} for MITC in all run-off scenarios with 97.5% run-off reduction following application to carrots (FOCUS-root vegetables)

Scenario	Waterbody	Max PEC _{SW} (µg/L)	Max PEC _{SED} (µg/kg)	1 day TWAEC _{SW} (µg/L)	2 day TWAEC _{SW} (µg/L)	4 day TWAEC _{SW} (µg/L)	7 day TWAEC _{SW} (µg/L)	14 day TWAEC _{SW} (µg/L)	21 day TWAEC _{SW} (µg/L)	28 day TWAEC _{SW} (µg/L)
R1	Pond	0.0071	0.0015	0.0055	0.0043	0.0028	0.0017	0.0010	0.0007	0.0005
R1	Stream	1.1090	0.0752	0.2590	0.1300	0.0648	0.0371	0.0286	0.0190	0.0143
R2	Stream	0.1620	0.0229	0.0897	0.0570	0.0293	0.0169	0.0085	0.0056	0.0042
R3	Stream	3.1860	0.2650	1.1650	0.5830	0.2920	0.1670	0.0838	0.0559	0.0419
R4	Stream	1.5490	0.2240	1.2210	0.6600	0.3310	0.1890	0.0947	0.0631	0.0473

Table B.9.2.16-6: Step 4 PEC_{SW} and PEC_{SED} for MITC in all run-off scenarios with 97.5% run-off reduction following application to corn salad (FOCUS-leafy vegetables)

Scenario	Waterbody	Max PEC _{SW} (µg/L)	Max PEC _{SED} (µg/kg)	1 day TWAEC _{SW} (µg/L)	2 day TWAEC _{SW} (µg/L)	4 day TWAEC _{SW} (µg/L)	7 day TWAEC _{SW} (µg/L)	14 day TWAEC _{SW} (µg/L)	21 day TWAEC _{SW} (µg/L)	28 day TWAEC _{SW} (µg/L)
R1	Pond	0.0031	0.0006	0.0023	0.0018	0.0011	0.0007	0.0004	0.0003	0.0003
R1	Stream	1.0340	0.1010	0.3970	0.1990	0.0995	0.0568	0.0302	0.0202	0.0151
R1	Pond	0.0110	0.0020	0.0083	0.0061	0.0036	0.0022	0.0012	0.0008	0.0006
R1	Stream	0.0831	0.0137	0.0585	0.0293	0.0146	0.0095	0.0048	0.0032	0.0024
R2	Stream	2.4020	0.2420	1.0400	0.5200	0.2600	0.1490	0.0804	0.0536	0.0402
R3	Stream	0.3360	0.0566	0.2900	0.1480	0.0743	0.0424	0.0212	0.0142	0.0106
R4	Stream	0.8930	0.1260	0.6240	0.3120	0.1560	0.0966	0.0485	0.0323	0.0242

Table B.9.2.16-7: Step 4 PEC_{SW} and PEC_{SED} for MITC in all run-off scenarios with 97.5% run-off reduction following application to tomatoes (FOCUS-fruitlegging vegetables)

Scenario	Waterbody	Max PEC _{SW} (µg/L)	Max PEC _{SED} (µg/kg)	1 day TWAEC _{SW} (µg/L)	2 day TWAEC _{SW} (µg/L)	4 day TWAEC _{SW} (µg/L)	7 day TWAEC _{SW} (µg/L)	14 day TWAEC _{SW} (µg/L)	21 day TWAEC _{SW} (µg/L)	28 day TWAEC _{SW} (µg/L)
R2	Stream	0.7820	0.1040	0.4600	0.2650	0.1340	0.0767	0.0384	0.0256	0.0192
R3	Stream	5.7780	0.5970	3.0450	1.5260	0.7640	0.4370	0.2330	0.1560	0.1170
R4	Stream	3.5220	0.2440	1.0130	0.5420	0.2710	0.1550	0.0783	0.0524	0.0393

Table B.9.2.16-8: Step 4 PEC_{SW} and PEC_{SED} for MITC in all run-off scenarios with 97.5% run-off reduction following application to potatoes (FOCUS-potatoes)

Scenario	Waterbody	Max PEC _{SW} (µg/L)	Max PEC _{SED} (µg/kg)	1 day TWAEC _{SW} (µg/L)	2 day TWAEC _{SW} (µg/L)	4 day TWAEC _{SW} (µg/L)	7 day TWAEC _{SW} (µg/L)	14 day TWAEC _{SW} (µg/L)	21 day TWAEC _{SW} (µg/L)	28 day TWAEC _{SW} (µg/L)
R1	Pond	0.0022	0.0004	0.0015	0.0011	0.0007	0.0005	0.0002	0.0002	0.0001
R1	Stream	0.3890	0.0391	0.1290	0.0876	0.0438	0.0258	0.0129	0.0086	0.0065
R2	Stream	0.0037	0.0007	0.0020	0.0014	0.0007	0.0004	0.0002	0.0001	0.0001
R3	Stream	0.0101	0.0015	0.0051	0.0028	0.0014	0.0008	0.0004	0.0003	0.0002

Risk assessment for aquatic organisms :

The risk assessment for aquatic organisms is based on the Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC of October 2002.

The assessment of the chronic risk to aquatic organisms is not justified since the DT₅₀ of MITC, whole water-sediment system is clearly below 2 days. The active substance is applied once a year. Moreover, soil sterilants are generally not used every year.

The assessment of the risk to sediment dwelling organisms is not justified since the properties of MITC (low K_{oc}, high solubility in water) exclude exposure of the sediment organisms. Moreover, a low level of MITC was observed transiently in the sediment phase of the w/s study (1.5-4.5 % AR in the sediment after 72 h).

Table B.9.2.16-9 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to the metabolite MITC for the intended use in carrot (1 x 408 kg a.s./ha) based on FOCUS step 3, laboratory soil degradation data

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer zone	PEC _{sw} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	D 3	ditch	<i>Oncorhynchus mykiss</i>	96 h	0.0531	1 m	0.2740	194	100
	D 6	ditch				1 m	1.6640	31.9	100
	R 1	pond				1 m	0.2840	187	100
	R 1	stream				1 m	43.2370	1.23	100
	R 2	stream (1 st crop)				1 m	5.2130	10.2	100
	R 3	stream				1 m	115.4920	0.46	100
	R 4	stream				1 m	49.7660	1.07	100
MITC	D 3	ditch	<i>Daphnia magna</i>	48 h	0.076	1 m	0.2740	277	100
	D 6	ditch				1 m	1.6640	45.7	100
	R 1	pond				1 m	0.2840	268	100
	R 1	stream				1 m	43.2370	1.76	100
	R 2	stream (1 st crop)				1 m	5.2130	14.6	100
	R 3	stream				1 m	115.4920	0.66	100
	R 4	stream				1 m	49.7660	1.53	100
MITC	D 3	ditch	<i>Pseudokirchneriella subcapitata</i>	72 h	0.28	1 m	0.2740	1022	10
	D 6	ditch				1 m	1.6640	168	10
	R 1	pond				1 m	0.2840	986	10
	R 1	stream				1 m	43.2370	6.48	10
	R 2	stream (1 st crop)				1 m	5.2130	53.7	10
	R 3	stream				1 m	115.4920	2.42	10
	R 4	stream				1 m	49.7660	5.63	10

MITC	D 3	ditch	<i>Lemna gibba</i>	7 d	0.59	1 m	0.2740	2153	10
	D 6	ditch				1 m	1.6640	355	10
	R 1	pond				1 m	0.2840	2077	10
	R 1	stream				1 m	43.2370	13.6	10
	R 2	stream (1 st crop)				1 m	5.2130	113	10
	R 3	stream				1 m	115.4920	5.11	10
	R 4	stream				1 m	49.7660	11.9	10

Table B.9.2.16-10 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to the metabolite MITC for the intended use in corn salad (1 x 306 kg a.s./ha) based on FOCUS step 3, laboratory soil degradation data

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{sw} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	D 3	ditch (1 st crop)	<i>Oncorhynchus mykiss</i>	96 h	0.0531	1 m	0.0002	>10000	100
	D 3	ditch (2 nd crop)				1 m	0.0000	>10000	100
	D 4	pond				1 m	0.0129	4116	100
	D 4	stream				1 m	0.4280	124	100
	D 6	ditch				1 m	0.0000	>10000	100
	R 1	pond (1 st crop)				1 m	0.1230	432	100
	R 1	stream (1 st crop)				1 m	40.7640	1.30	100
	R 1	pond (2 nd crop)				1 m	0.4290	124	100
	R 1	stream (2 nd crop)				1 m	2.6630	19.9	100
	R 2	stream (2 nd crop)				1 m	78.4160	0.68	100
	R 3	stream (2 nd crop)				1 m	10.7750	4.93	100
	R 4	stream (2 nd crop)				1 m	28.6220	1.86	100

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	D 3	ditch (1 st crop)	<i>Daphnia magna</i>	48 h	0.076	1 m	0.0002	>10000	100
	D 3	ditch (2 nd crop)				1 m	0.0000	>10000	100
	D 4	pond				1 m	0.0129	5891	100
	D 4	stream				1 m	0.4280	178	100
	D 6	ditch				1 m	0.0000	>10000	100
	R 1	pond (1 st crop)				1 m	0.1230	618	100
	R 1	stream (1 st crop)				1 m	40.7640	1.86	100
	R 1	pond (2 nd crop)				1 m	0.4290	177	100
	R 1	stream (2 nd crop)				1 m	2.6630	28.5	100
	R 2	stream (2 nd crop)				1 m	78.4160	0.97	100
	R 3	stream (2 nd crop)				1 m	10.7750	7.05	100
	R 4	stream (2 nd crop)				1 m	28.6220	2.66	100
MITC	D 3	ditch (1 st crop)	<i>Pseudokirchneriella subcapitata</i>	72 h	0.28	1 m	0.0002	>10000	10
	D 3	ditch (2 nd crop)				1 m	0.0000	>10000	10
	D 4	pond				1 m	0.0129	>10000	10
	D 4	stream				1 m	0.4280	654	10
	D 6	ditch				1 m	0.0000	>10000	10
	R 1	pond (1 st crop)				1 m	0.1230	2276	10
	R 1	stream (1 st crop)				1 m	40.7640	6.87	10

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
	R 1	pond (2 nd crop)				1 m	0.4290	633	10
	R 1	stream (2 nd crop)				1 m	2.6630	105	10
	R 2	stream (2 nd crop)				1 m	78.4160	3.57	10
	R 3	stream (2 nd crop)				1 m	10.7750	26.0	10
	R 4	stream (2 nd crop)				1 m	28.6220	9.78	10
MITC	D 3	ditch (1 st crop)	<i>Lemna gibba</i>	7 d	0.59	1 m	0.0002	>10000	10
	D 3	ditch (2 nd crop)				1 m	0.0000	>10000	10
	D 4	pond				1 m	0.0129	>10000	10
	D 4	stream				1 m	0.4280	1379	10
	D 6	ditch				1 m	0.0000	>10000	10
	R 1	pond (1 st crop)				1 m	0.1230	4797	10
	R 1	stream (1 st crop)				1 m	40.7640	14.5	10
	R 1	pond (2 nd crop)				1 m	0.4290	1375	10
	R 1	stream (2 nd crop)				1 m	2.6630	222	10
	R 2	stream (2 nd crop)				1 m	78.4160	7.52	10
	R 3	stream (2 nd crop)				1 m	10.7750	54.8	10

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
	R 4	stream (2 nd crop)				1 m	28.6220	20.6	10

Table B.9.2.16-11 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to the metabolite MITC for the intended use in tomato (1 x 612 kg a.s./ha) based on FOCUS step 3, laboratory soil degradation data

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	D 6	ditch	<i>Oncorhynchus mykiss</i>	96 h	0.0531	1 m	0.8410	63.1	100
	R 2	stream				1 m	25.0930	2.12	100
	R 3	stream				1 m	184.6650	0.29	100
	R 4	stream				1 m	114.8120	0.46	100
MITC	D 6	ditch	<i>Daphnia magna</i>	48 h	0.076	1 m	0.8410	90.4	100
	R 2	stream				1 m	25.0930	3.03	100
	R 3	stream				1 m	184.6650	0.41	100
	R 4	stream				1 m	114.8120	0.66	100
MITC	D 6	ditch	<i>Pseudokirchneriella subcapitata</i>	72 h	0.28	1 m	0.8410	333	10
	R 2	stream				1 m	25.0930	11.2	10
	R 3	stream				1 m	184.6650	1.52	10
	R 4	stream				1 m	114.8120	2.44	10
MITC	D 6	ditch	<i>Lemna gibba</i>	7 d	0.59	1 m	0.8410	702	10
	R 2	stream				1 m	25.0930	23.5	10
	R 3	stream				1 m	184.6650	3.19	10
	R 4	stream				1 m	114.8120	5.14	10

Table B.9.2.16-12 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to the metabolite MITC for the intended use in potato (1 x 153 kg a.s./ha) based on FOCUS step 3, laboratory soil degradation data

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	D 3	ditch	<i>Oncorhynchus mykiss</i>	96 h	0.0531	1 m	0.0319	1665	100
	D 4	pond				1 m	0.5980	88.8	100
	D 4	stream				1 m	3.3540	15.8	100
	D 6	ditch (1 st crop)				1 m	0.0000	>10000	100
	D 6	ditch (2 nd crop)				1 m	9.0840	5.85	100
	R 1	pond				1 m	0.0858	619	100
	R 1	stream				1 m	13.0580	4.07	100
	R 2	stream				1 m	0.1190	446	100
	R 3	stream				1 m	0.3280	162	100
MITC	D 3	ditch	<i>Daphnia magna</i>	48 h	0.076	1 m	0.0319	2382	100
	D 4	pond				1 m	0.5980	127	100
	D 4	stream				1 m	3.3540	22.7	100
	D 6	ditch (1 st crop)				1 m	0.0000	>10000	100
	D 6	ditch (2 nd crop)				1 m	9.0840	8.37	100
	R 1	pond				1 m	0.0858	886	100
	R 1	stream				1 m	13.0580	5.82	100
	R 2	stream				1 m	0.1190	639	100
	R 3	stream				1 m	0.3280	232	100
MITC	D 3	ditch	<i>Pseudokirchneriella subcapitata</i>	72 h	0.28	1 m	0.0319	8777	10
	D 4	pond				1 m	0.5980	468	10
	D 4	stream				1 m	3.3540	83.5	10
	D 6	ditch (1 st crop)				1 m	0.0000	>10000	10
	D 6	ditch (2 nd crop)				1 m	9.0840	30.8	10
	R 1	pond				1 m	0.0858	3263	10
	R 1	stream				1 m	13.0580	21.4	10
	R 2	stream				1 m	0.1190	2353	10
	R 3	stream				1 m	0.3280	854	10
	D 3	ditch	<i>Lemna gibba</i>	7 d	0.59	1 m	0.0319	>10000	10
	D 4	pond				1 m	0.5980	987	10

	D 4	stream				1 m	3.3540	176	10
	D 6	ditch (1 st crop)				1 m	0.0000	>10000	10
	D 6	ditch (2 nd crop)				1 m	9.0840	64.9	10
	R 1	pond				1 m	0.0858	6876	10
	R 1	stream				1 m	13.0580	45.2	10
	R 2	stream				1 m	0.1190	4958	10
	R 3	stream				1 m	0.3280	1799	10

The TER values based on FOCUS step 3 calculations indicate that refinement of the PEC_{SW} values is needed.

Table B.9.2.16-13 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to the metabolite MITC for the intended use in carrot (1 x 408 kg a.s./ha) based on FOCUS step 4, laboratory soil degradation data

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	R 1	pond	<i>Oncorhynchus mykiss</i>	96 h	0.0531	20 m	0.0071	7479	100
	R 1	stream				20 m	1.1090	47.9	100
	R 2	stream				20 m	0.1620	328	100
	R 3	stream				20 m	3.1860	16.7	100
	R 4	stream				20 m	1.5490	34.3	100
MITC	R 1	pond	<i>Daphnia magna</i>	48 h	0.076	20 m	0.0071	>10000	100
	R 1	stream				20 m	1.1090	68.5	100
	R 2	stream				20 m	0.1620	469	100
	R 3	stream				20 m	3.1860	23.9	100
	R 4	stream				20 m	1.5490	49.1	100
MITC	R 1	pond	<i>Pseudokirchneriella subcapitata</i>	72 h	0.28	20 m	0.0071	>10000	10
	R 1	stream				20 m	1.1090	252	10
	R 2	stream				20 m	0.1620	1728	10
	R 3	stream				20 m	3.1860	87.9	10
	R 4	stream				20 m	1.5490	181	10
MITC	R 1	pond	<i>Lemna gibba</i>	7 d	0.59	20 m	0.0071	>10000	10
	R 1	stream				20 m	1.1090	532	10
	R 2	stream				20 m	0.1620	3642	10
	R 3	stream				20 m	3.1860	185	10
	R 4	stream				20 m	1.5490	381	10

Table B.9.2.16-14 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to the metabolite MITC for the intended use in corn salad (1 x 306 kg a.s./ha) based on FOCUS step 4, laboratory soil degradation data

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	R 1	pond (1 st crop)	<i>Oncorhynchus mykiss</i>	96 h	0.0531	20 m	0.0031	>10000	100
	R 1	stream (1 st crop)				20 m	1.0340	51.4	100
	R 1	pond (2 nd crop)				20 m	0.0110	4827	100
	R 1	stream (2 nd crop)				20 m	0.0831	639	100
	R 2	stream (2 nd crop)				20 m	2.4020	22.1	100
	R 3	stream (2 nd crop)				20 m	0.3360	158	100
	R 4	stream (2 nd crop)				20 m	0.8930	59.5	100
MITC	R 1	pond (1 st crop)	<i>Daphnia magna</i>	48 h	0.076	20 m	0.0031	>10000	100
	R 1	stream (1 st crop)				20 m	1.0340	73.5	100
	R 1	pond (2 nd crop)				20 m	0.0110	6909	100
	R 1	stream (2 nd crop)				20 m	0.0831	915	100
	R 2	stream (2 nd crop)				20 m	2.4020	31.6	100
	R 3	stream (2 nd crop)				20 m	0.3360	226	100
	R 4	stream (2 nd crop)				20 m	0.8930	85.1	100
MITC	R 1	pond (1 st crop)	<i>Pseudokirchneriella subcapitata</i>	72 h	0.28	20 m	0.0031	>10000	10
	R 1	stream (1 st crop)				20 m	1.0340	271	10
	R 1	pond (2 nd crop)				20 m	0.0110	>10000	10
	R 1	stream (2 nd crop)				20 m	0.0831	3369	10

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
	R 2	stream (2 nd crop)				20 m	2.4020	117	10
	R 3	stream (2 nd crop)				20 m	0.3360	833	10
	R 4	stream (2 nd crop)				20 m	0.8930	314	10
MITC	R 1	pond (1 st crop)	<i>Lemna gibba</i>	7 d	0.59	20 m	0.0031	>10000	10
	R 1	stream (1 st crop)				20 m	1.0340	571	10
	R 1	pond (2 nd crop)				20 m	0.0110	>10000	10
	R 1	stream (2 nd crop)				20 m	0.0831	7100	10
	R 2	stream (2 nd crop)				20 m	2.4020	246	10
	R 3	stream (2 nd crop)				20 m	0.3360	1756	10
	R 4	stream (2 nd crop)				20 m	0.8930	661	10

Table B.9.2.16-15 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to the metabolite MITC for the intended use in tomato (1 x 612 µg a.s./ha) based on FOCUS step 4, laboratory soil degradation data

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{SW} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	R 2	stream	<i>Oncorhynchus mykiss</i>	96 h	0.0531	20 m	0.7820	67.9	100
	R 3	stream				20 m	5.7780	9.19	100
	R 4	stream				20 m	3.5220	15.1	100
MITC	R 2	stream	<i>Daphnia magna</i>	48 h	0.076	20 m	0.7820	97.2	100
	R 3	stream				20 m	5.7780	13.2	100
	R 4	stream				20 m	3.5220	21.6	100
MITC	R 2	stream	<i>Pseudokirchneriella subcapitata</i>	72 h	0.28	20 m	0.7820	358	10
	R 3	stream				20 m	5.7780	48.5	10
	R 4	stream				20 m	3.5220	79.5	10
MITC	R 2	stream	<i>Lemna gibba</i>	7 d	0.59	20 m	0.7820	754	10

	R 3	stream				20 m	5.7780	102	10
	R 4	stream				20 m	3.5220	168	10

Table B.9.2.16-16 : Toxicity Exposure Ratio's (TER's) for aquatic organisms exposed to the metabolite MITC for the intended use in potato (1 x 153 kg a.s./ha) based on FOCUS step 4, laboratory soil degradation data

Test substance	Scenario	Water body type	Test species	Time-scale	Endpoint (mg a.s./L)	Buffer-zone	PEC _{sw} , initial (µg a.s./L)	TER	Annex VI Trigger value
MITC	R 1	pond	<i>Oncorhynchus mykiss</i>	96 h	0.0531	20 m	0.0022	>10000	100
	R 1	stream				20 m	0.3890	137	100
	R 2	stream				20 m	0.0037	>10000	100
	R 3	stream				20 m	0.0101	5257	100
MITC	R 1	pond	<i>Daphnia magna</i>	48 h	0.076	20 m	0.0022	>10000	100
	R 1	stream				20 m	0.3890	195	100
	R 2	stream				20 m	0.0037	>10000	100
	R 3	stream				20 m	0.0101	7525	100
MITC	R 1	pond	<i>Pseudokirchneriella subcapitata</i>	72 h	0.28	20 m	0.0022	>10000	10
	R 1	stream				20 m	0.3890	720	10
	R 2	stream				20 m	0.0037	>10000	10
	R 3	stream				20 m	0.0101	>10000	10
MITC	R 1	pond	<i>Lemna gibba</i>	7 d	0.59	20 m	0.0022	>10000	10
	R 1	stream				20 m	0.3890	1517	10
	R 2	stream				20 m	0.0037	>10000	10
	R 3	stream				20 m	0.0101	>10000	10

Conclusions :

The uses in greenhouse pose no risk to aquatic organisms.

FOCUS step 4 scenario, buffer zone of 20 m, carrot (1 x 408 kg a.s./ha) :

TER values for fish do not pass the trigger in 3 out of 5 scenarios (R1 p and R2 s acceptable)

TER values for aquatic invertebrates do not pass the trigger in 3 out of 5 scenarios (R1 p and R2 s acceptable)

The risk to algae and aquatic plants is acceptable for all scenarios

FOCUS step 4 scenario, buffer zone of 20 m, corn salad (1 x 306 kg a.s./ha) :

TER values for fish do not pass the trigger in 3 out of 7 scenarios (R1 p, R1 s (2nd crop), R3 s acceptable)

TER values for aquatic invertebrates do not pass the trigger in 3 out of 7 scenarios (R1 p, R1 s (2nd crop), R3 s acceptable)

The risk to algae and aquatic plants is acceptable for all scenarios

FOCUS step 4 scenario, buffer zone of 20 m, tomatoes (1 x 612 kg a.s./ha) :

TER values for fish do not pass the trigger in 3 out of 3 scenarios

TER values for aquatic invertebrates do not pass the trigger in 3 out of 3 scenarios

The risk to algae and aquatic plants is acceptable for all scenarios

FOCUS step 4 scenario, buffer zone of 20 m, potatoes (1 x 153 kg a.s./ha) :

The risk to fish, aquatic invertebrates, algae and aquatic plants is acceptable for all scenarios

Some additional step 3 scenarios are also acceptable.

As already mentioned in the section on fate and behaviour, the PEC_{sw} values should be used with precaution. The FOCUS sw models were not developed with soil fumigants in mind. The models do not properly consider the volatilisation of MITC and therefore highly overestimate the actual surface water contamination.

In conclusion, the risk of metam-sodium and its metabolite MITC to aquatic organisms is acceptable for the intended uses with a buffer zone of 20 m.

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

Revised in August 2010:

The ecotoxicologically relevant endpoints for mammals were derived from the section on mammalian toxicology.

Table B.9.3-1 : Summary of effects of metam-sodium and its major metabolite MITC on mammals

Test species	Test substance	Test system	Endpoints	References
rat	metam-sodium	acute oral toxicity	LD ₅₀ = 896 mg a.s./kg b.w.	Baldrick and Healing, 1991a
	MITC		LD ₅₀ = 147 mg MITC/kg b.w.	Kieczka and Kirsch, 1986
rat	metam-sodium	2-generation reproduction toxicity	NOAEL = 1.5 mg a.s./kg b.w./day	Allen, 1991 Rattray, 1994
	MITC		NOAEL = 0.44 mg MITC/kg b.w./day	Brown, 1984 Brown, 1988 Iwan, Kaiser, and Hollihn, 1988

Added in August 2010:

Small mammal survey on sterilized and unsterilized carrot plots and at the field perimeter in a commercial arable field in Les Landes, France during spring 2008. (Bakker F., Reich T., 2010).

Aim

The aim of this study was to propose a list of small mammal species in selected carrot field that can be addressed as focal small mammal species in a refined risk assessment for plant protection products.

Objectives

The objectives of this study were to determine whether the presence of the small mammal species and their frequency is related to the metam sodium treatment, both in time (before and after treatment) as in space (comparing control, reference and test item treated plots).

Guidelines :

Not applicable

GLP :

Not applicable

Materials and Methods :**Study area :**

The experimental site was a 12 ha field in Les Landes (France), which is a major area for carrot production. Within this area, 8 square plots of 1 ha each and 4 more rectangular plots with 1 rounded side were established. The field borders had only little vegetation that provided the same or less cover for rodents as the surrounding meadows. These meadows were grazed by horses during the sampling period, resulting in short grass at all times. The north border and partially the south and south-east border of the field were neighboured by woodland and shrubbery.

Method and parameters :

Two of these plots were not cultivated at all. The remaining plots were tilled on 3 April 2008, subjected to injection on 8 and 9 April 2008, ploughed on 29 April 2008 (including butt formation) and subsequently sown with carrot on May 5th, 2008. In four of these plots injection was with metam-sodium. The other six were injected with water only. Two of the water plots were subsequently treated with a broad spectrum insecticide, registered for sterilization (Pyrinex, a.i. chlorpyrifos).

Small mammal surveys were performed during four periods in the spring of 2008. Longworth live traps were placed in and around the field before cultivation, shortly after application of the sterilant, after sowing and two months after application. During the study period there was little or no vegetation on the land.

The field was visited two times after sunset with a bat detector.

Data recording and analysis :

Of the trapped animals, the species and sex were identified; they were marked for recapture purposes. The marking signs indicated the treatment of the field they were trapped in.

Findings :

The captures were not spatially homogeneous; the highest rate of captured mice per trap was observed near woodland and shrubbery.

The woodmouse (*Apodemus sylvaticus*) was the most frequently trapped species. The vole (*Microtus* spp.) was the second species trapped in a much lower frequency. About 78 individuals of the woodmouse versus 10 vole individuals were captured during the trial.

During two bat observations rounds, each time only one bat (*Pipistrellus* spp.) could be heard during the whole walk through all the plots.

Although there seemed to be a trend that mice captured in the field were also mostly recaptured in the field, numbers caught were too low for a systematic analysis. However, mice caught in water plots were recaptured in sterilized plots and vice versa.

Important is that the population inside the field was obviously much smaller to non-existent. This habitat avoidance seems unrelated to cultivation or treatment as it already occurred before the study started. A possible explanation is that the mice avoid the open field due of the missing protection from predatory birds that were numerous in the surrounding woods. The cattle present in the field over winter may provide an additional explanation.

Conclusions :

The woods and shrubs surrounding the experimental site were inhabited by a population of about 50-60 wood mice (*Apodemus sylvaticus*) and probably around 10 voles (*Microtus*) that were attracted to the traps. In the field, however, no voles were caught and only five wood mice. This obvious avoidance of the open field was already clear before the cultivation started. The same applies to bats, where a large population was observed in the woods, but only one *Pipistrellus* was heard over the experimental plots. Exposure of these small mammals to metam-sodium, either directly or indirectly through food seems therefore rather limited.

Conclusions of the RMS :

The study is considered acceptable and is taken into account in the mammalian risk assessment.

Added in August 2010:

The level of MITC in arthropods sampled in a field treated with metam-sodium was determined analytically.

METAM SODIUM: A field study to determine residues in carrot field invertebrates as potential food items for birds and small mammals in SW France. (Bakker F. & Roig J., 2010).

See study summary under B.9.1.7.

First tier risk assessment for mammals :

The risk assessment for mammals is based on the Guidance Document for birds and mammals under Council Directive 91/414/EEC of November 2002. As a worst case it was assumed that the mammals obtained 100 % of their diet in the treated area.

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation **under a plastic film** on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation **under a plastic film** on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation **under a plastic film** on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation **under a plastic film** on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil; the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is compressed with a roller. After a waiting period of 2 to 4 weeks, the soil is cultivated to allow the non-mineralised gasses to disperse. Since the product is phytotoxic, it is advisable to perform a germination test (Cress-test) on samples of soils collected from the treated field, before planting. Mammals are not exposed to the uses in greenhouse.

Added in August 2010:

1 - Risk assessment presented by the notifier:

Acute risk assessment of Metam-Sodium on mammals.

EFSA have requested an acute risk assessment for metam-sodium for insectivorous mammals that might feed on freshly treated bare soil fields.

Based on the physico-chemical characteristics and toxicological endpoints of metam sodium it is not meaningful to calculate the risk assessment for the parent active substance but instead for the primary metabolite MITC.

The degradation rate of metam-sodium was assessed in four different soil types, the mean DT₅₀ at 20°C was 10 minutes, (minimum - maximum DT₅₀ = 4 – 17 minutes), whereas the DT₉₀ is set to be 34 minutes (minimum - maximum DT₉₀ = 13 – 57 minutes). MITC is the only relevant metabolite of metam-sodium (Hall, B.E. *et al.* 2004).

The toxicity of metam-sodium and MITC to mammals has been assessed in acute and long term toxicity tests. The relevant toxicological endpoints of metam-sodium and MITC are presented in the table below. Comparing these endpoints we conclude that for both acute and long term exposure MITC is more toxic to mammals than metam-sodium.

Table B.9.3-2 : The relevant toxicological endpoints of metam-sodium and MITC for mammals

	metam-sodium (mg/kg bw/day)	References	MITC (mg/kg bw/day)	References
Acute mammalian LD ₅₀	Rat 896	Baldrick and Healing, 1991a	Rat 147 Mouse 100	Kieczka and Kirsch, 1986 Suggested by EFSA based on Dazomet dossier
Mammalian Long term NOEC	Rat 1.5	Allen, 1991, Rattray, 1994	Rat 0.44	Brown, 1984; Brown, 1988; Iwan, Kaiser and Hollihn, 1998

Conclusion:

Based on the very fast degradation of metam-sodium into the more toxic MITC we consider that when the acute risk of MITC is acceptable for mammals, the risk of metam-sodium will also be acceptable.

Acute risk assessment of MITC on mammals.

The Acute Risk to Birds and Mammals potentially exposed following proposed commercial use of Metam Sodium. (Brown K.C., 2010). Unpublished report

Summary:

Glasshouse uses of metam sodium are excluded from this risk assessment since there will be no exposure to mammals from closed crop systems. The field uses which distribute MITC widely throughout the soil, injection/rotavation, injection/coverage and drip irrigation are considered to present the greatest risk to mammals, since the majority of the soil dwelling arthropods and earthworms will receive almost uniform exposure.

The toxicity of MITC to mammals has been assessed in acute tests. The value of 100 mg/kg bw/d (mouse) was suggested by EFSA.

Acute risk assessment for insectivorous mammals

A field survey of mammals at a metam sodium treated site (Bakker and Reich, 2010) found extremely low numbers of mammals in field. Only the wood mouse *Apodemus sylvaticus* was caught in field and this is considered to be the focal species for this risk assessment. Gurney *et al* (1998) consider that the woodmouse has a mean body weight of 18g.

Specimens of invertebrates were collected from a field study with metam sodium (see IIIA 10.1.4). The initial residue mean value was 7.3 mg/kg. This gives equal weight to the values derived from each of the three dates. It is proposed to use a multiplier of 2.12 so as to estimate a worst case 90th centile for the MITC residue in insects as food items for omnivorous mammals of 15.48 mg/kg at an equivalent MITC application rate of 346 kg as/ha (resulting in an RUD (Residue per unit Dose) value of 0.045 mg/kg at a rate of 1 kg MITC/ha).

Woodmouse has been selected as the appropriate focal species of insect feeding mammal for bare soil treated with metam sodium. Woodmouse are omnivorous, their diet is highly dependent on habitat. A value of PD of 0.16 is used in this risk assessment.

Woodmouse has a mean body weight of 18 g (Gurney *et al*, 1998). The daily consumption for an 18 g mammal feeding entirely on soil invertebrates has been estimated using the PSD (now CRD) dietary calculator tool as 21.26 g/day which gives and the FIR/bw value of 1.18.

$$\text{ETE} = (\text{FIR/bw}) * C * \text{AV} * \text{PT} * \text{PD}$$

AV and PT are set at 1 for this risk assessment but PD is 0.16.

Table B.9.3-3 : Acute risk assessment for insectivorous mammals in field crops

Field uses of metam sodium	MITC rate assuming complete conversion from metam sodium* (kg as/ha)	RUD MITC	FIR/bw Woodmouse	PD	ETE	TER
Carrots	230 (408)	0.045	1.18	0.16	1.95	51
Corn Salad	173 (306)	0.045	1.18	0.16	1.47	68
Potato	86.3 (153)	0.045	1.18	0.16	0.73	137

* metam sodium application rate in brackets

The TER values derived for use in all field crop applications are above the threshold of 10 and indicate acceptable acute risk to mammals where soil invertebrates comprise up to 16% of the diet.

Acute risk assessment for mammals feeding on earthworms

A survey of mammals at a metam sodium treated site in France (Bakker and Reich, 2010) found extremely low numbers of mammals in fields. Only the wood mouse *Apodemus sylvaticus* was caught in field. Gurney *et al*, (1998) consider that the woodmouse has a body weight of 18 g.

Wood mice are omnivorous, their diet is highly dependent on habitat. To present the worst case risk a figure of 40% has been used in the following calculations.

Estimated Residues in Earthworms

The BCF formula in SANCO 4145/2000 is not considered to be appropriate due to the extremely short half life of MITC of 2.1 days. The use of BCF assumes residues are acquired over a longer period of time than would be appropriate for this acute risk assessment.

As residue data on earthworms are not available, it is proposed to use the measured fresh residues for invertebrates as a surrogate.

Risk Assessment for mammals feeding on earthworms

Wood mouse has been selected as the appropriate focal species of worm feeding mammal for bare soil treated with metam sodium. Gurney *et al*, (1998) consider that the woodmouse has a body weight of 18 g. SANCO 4145/2000 gives a FIR/bw for worm feeding mammals of 1.4.

$$\text{ETE} = (\text{FIR/bw}) * C * \text{AV} * \text{PT} * \text{PD}$$

It is assumed that wood mice obtain all their food from the treated area, however, PD is set at 0.4 to reflect the maximum contribution that earthworms can make to the diet of woodmouse. AV and PT are set at 1.

The lowest acute LD₅₀ value for MITC is 100 mg/kg (mouse).

Table B.9.3-4 : Acute risk assessment for worm feeding mammals in field crops

Field uses of metam sodium	MITC rate assuming complete conversion from metam sodium* (kg as/ha)	RUD MITC	FIR/bw Woodmouse	PD	ETE	TER
Carrots	230 (408)	0.046	1.4	0.4	5.9	17.0
Corn Salad	173 (306)	0.046	1.4	0.4	4.5	22.2
Potato	86.3 (153)	0.046	1.4	0.4	2.2	45.5

* metam sodium application rate in brackets

The TER values derived for use in carrots, corn salad and potatoes are above the threshold of 10 and the acute risk to worm feeding mammals is considered to be acceptable.

2 – Risk assessment performed by RMS:

Acute toxicity endpoint MITC:

EFSA suggested to use the acute toxicity endpoint for MITC from the dossier of dazomet, since this is the worst case.

LD₅₀ (female mouse) = 100 mg MITC/kg b.w. (Kirsch, 1987b)

Focal species

From the field survey of mammals (Bakker F., Reich T., 2010) only the wood mouse *Apodemus sylvaticus* was caught in the field. RMS agrees with the notifier to consider the wood mouse for the mammalian risk assessment.

Measured residues in food items:

From the residue field study (Bakker F. & Roig J., 2010) residues of MITC in **invertebrates** were determined after 24, 48 and 72 hours after treatment of metam sodium at 612 kg a.s./ha (equivalent to 346 kg MITC/ha). The residue was 4.0 mg/kg (mean of 2 plots) after 24 hours, 13.3 mg/kg (mean of 2 plots) after 48 hours and 4.8 mg/kg (mean of 4 plots) after 72 hours. An initial mean residue value over 0-72 hours was calculated as 7.3 mg/kg.

For the acute risk assessment 90th percentiles are needed. From the EFSA Guidance Document on Birds and Mammals (Appendix E), mean RUD and 90th RUD are compared for ground dwelling invertebrates on bare soil. RUD 90th is 13.8 mg/kg and mean RUD is 7.5 leading to a factor 1.84. This is in agreement with the proposal of the notifier to use a factor 1.85 – 2.17. Taking the worst case factor of 2.17, the 90th residue value is calculated 16 mg/kg for an application rate of 346 kg MITC/ha. Recalculation in RUD (residue per unit dose) results in 0.046 mg/kg for an application rate of 1 kg MITC/ha.

For the residue data in **earthworms** it is not appropriate to use the BCF formula, due to the extremely short half-life of MITC of 2.1 days. In absence of residue field data obtained for earthworms, RMS agrees with the notifier to use the residue values in insects as surrogate.

Risk assessment for insectivorous mammals

The wood mouse is selected as the appropriate focal species for insectivorous mammals feeding on bare soil treated with metam-sodium. The wood mouse has a mean body weight of 18 g (Gurney *et al.*, 1998).

$$\text{Log (DEE)} = 0.8459 + (0.7050 \times (\log 18)) = 1.73$$

$$\text{DEE} = 54 \text{ kJ}$$

Table B.9.3-5 : Calculation of the ratio of food intake rate of soil invertebrates to body weight for a wood mouse per day

Food type	Energy content* (kJ/g dw)	Moisture content* (%)	Energy content (kJ/g ww)	Assimilation efficiency ** (%)	Yield (kJ/g ww)	Fresh food intake (g/day)	FIR/bw
soil invertebrates	19.3	84.6	2.97	85	2.53	21.30	1.18

* Value taken from Table 3 in Appendix I of SANCO/4145/2000

** Value taken from Table 5 in Appendix I of SANCO/4145/2000

For the first tier calculation it is assumed that the wood mice are obtaining all their food from the treated area and therefore AV, PD and PT are set at 1.

The greenhouse uses of metam-sodium are excluded from the risk assessment since there will be no exposure to mammals from closed crop systems.

The use of metam-sodium in grapes at vine planting is a very localized treatment and therefore the risk will be much lower and will be covered by the other field uses.

The field uses which distribute MITC widely throughout the soil, injection/rotavation, injection/coverage and drip irrigation are considered to present the greatest risk to mammals, since the majority of the soil dwelling invertebrates will receive almost uniform exposure. These crops are carrot, corn salad and potato (injection/rotavation), tomato (injection/coverage) and strawberry (drip irrigation).

Table B.9.3-6 : Acute risk assessment for insectivorous mammals eating contaminated soil invertebrates on bare soil for the use in field crops (based on endpoint $\text{LD}_{50} = 100 \text{ mg MITC/kg bw}$)

Field uses of metam-sodium	MITC rate assuming complete conversion from metam-sodium* (kg/ha)	FIR/bw	RUD	ETE	TER	Annex VI Trigger value
strawberry	346 (612)	1.18	0.046	18.8	5.32	10
tomato	346 (612)	1.18	0.046	18.8	5.32	10
carrot	230 (408)	1.18	0.046	12.5	8.01	10
corn salad	173 (306)	1.18	0.046	9.39	10.6	10
potato	86.3 (153)	1.18	0.046	4.68	21.3	10

metam-sodium application rate in brackets

Based on first tier assumptions (PD, PT = 1) and initial measured residues, the TER values for corn salad and potato indicate acceptable risk for insectivorous mammals. The TER values for strawberry, tomato and carrot are below the trigger value of 10.

Refinement of the PD value is proposed. Wood mice are omnivorous species, their diet is highly dependent on habitat. The Swedish Chemicals Inspectorate (KEMI, 2006) summarised the dietary composition of wood mouse in set-aside, winter wheat and intensive arable habitat, specifically for risk assessment purposes. In set-aside, the highest insect component throughout the year was 13% and in cereals it was 16%. A value of PD of 0.16 is therefore used in this risk assessment.

Table B.9.3-7 : Acute risk assessment for insectivorous mammals eating contaminated soil invertebrates on bare soil for the use in field crops (based on endpoint LD₅₀ = 100 mg MITC/kg bw and PD = 0.16)

Field uses of metam-sodium	MITC rate assuming complete conversion from metam-sodium* (kg/ha)	FIR/bw	RUD	PD	ETE	TER	Annex VI Trigger value
strawberry	346 (612)	1.18	0.046	0.16	3.00	33	10
tomato	346 (612)	1.18	0.046	0.16	3.00	33	10
carrot	230 (408)	1.18	0.046	0.16	2.00	50	10
corn salad	173 (306)	1.18	0.046	0.16	1.50	67	10
potato	86.3 (153)	1.18	0.046	0.16	0.75	133	10

* metam-sodium application rate in brackets

Based on PD = 0.16 and initial measured residues, the acute TER values for all field uses meet the trigger value of 10 for insectivorous mammals.

Risk assessment for vermivorous mammals

The wood mouse is selected as the appropriate focal species for mammals feeding on earthworms on bare soil treated with metam-sodium. The body weight of the wood mouse is 18 g (Gurney *et al.*, 1998). The FIR/bw value of 1.4 was used according to SANCO 4145/2000.

For the first tier calculation it is assumed that the wood mice are obtaining all their food from the treated area and therefore AV, PD and PT are set at 1.

Table B.9.3-8 : Acute risk assessment for vermivorous mammals eating contaminated earthworms on bare soil for the use in field crops (based on endpoint LD₅₀ = 100 mg MITC/kg bw)

Field uses of metam-sodium	MITC rate assuming complete conversion from metam-sodium* (kg/ha)	FIR/bw	RUD	ETE	TER	Annex VI Trigger value
strawberry	346 (612)	1.4	0.046	22.3	4.49	10
tomato	346 (612)	1.4	0.046	22.3	4.49	10
carrot	230 (408)	1.4	0.046	14.8	6.75	10
corn salad	173 (306)	1.4	0.046	11.1	8.98	10
potato	86.3 (153)	1.4	0.046	5.56	18.0	10

* metam-sodium application rate in brackets

Based on first tier assumptions (PD, PT = 1) and initial measured residues, the TER values for potato indicate acceptable risk for vermivorous mammals. The TER values for strawberry, tomato, carrot and corn salad are below the trigger value of 10.

Refinement of the PD value is proposed. Wood mice are omnivorous, their diet is highly dependent on habitat. The Swedish Chemicals Inspectorate (KEMI, 2006) summarised the dietary composition of wood mouse in set-aside, winter wheat and intensive arable habitat specifically for risk assessment purposes. In set-aside and winter wheat earthworms were not a component of the wood mouse diet. In intensive arable land the extent to which earthworms contributed to the diet was extremely seasonal, being 26% in April, 40% in May, 9% in June, zero in July, 5% in August and 13% in September. To present the worst case risk, a value of PD of 0.40 is used in this risk assessment.

Table B.9.3-9 : Acute risk assessment for vermivorous mammals eating contaminated earthworms on bare soil for the use in field crops (based on endpoint LD₅₀ = 100 mg MITC/kg bw and PD = 0.40)

Field uses of metam-sodium	MITC rate assuming complete conversion from metam-sodium* (kg/ha)	FIR/bw	RUD	PD	ETE	TER	Annex VI Trigger value
strawberry	346 (612)	1.4	0.046	0.40	8.91	11	10
tomato	346 (612)	1.4	0.046	0.40	8.91	11	10
carrot	230 (408)	1.4	0.046	0.40	5.92	17	10
corn salad	173 (306)	1.4	0.046	0.40	4.46	22	10
potato	86.3 (153)	1.4	0.046	0.40	2.22	45	10

* metam-sodium application rate in brackets

Based on PD = 0.40 and initial measured residues, the acute TER values for all field uses meet the trigger value of 10 for vermivorous mammals.

In conclusion, the risk of metam-sodium and its metabolite MITC to mammals is acceptable for the uses in greenhouse and localised use in vines. The risk is acceptable for the intended field uses in potato and corn salad (application rates 153 and 306 kg a.s./ha) for insectivorous mammals based on first tier assumptions (PD = 1). The risk is acceptable for the intended field uses in carrot, strawberry and tomato (application rates 408 and 612 kg a.s./ha) for insectivorous mammals based on refinement (PD = 0.16). The risk is acceptable for the intended field use in potato (application rate 153 kg a.s./ha) for vermivorous mammals based on first tier assumptions (PD = 1). The risk is acceptable for the intended field uses in corn salad, carrot, strawberry and tomato (application rates 306, 408 and 612 kg a.s./ha) for vermivorous mammals based on refinement (PD = 0.40).

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

B.9.4.1 Acute toxicity to bees (Annex IIA 8.3.1.1)

B.9.4.2 Bee brood feeding test (Annex IIA 8.3.1.2)

B.9.4.3 Acute toxicity of the preparations to bees (Annex IIIA 10.4.1)

B.9.4.4. Effects on bees of residues on crops (Annex IIIA 10.4.2)

B.9.4.5 Cage tests (Annex IIIA 10.4.3)

B.9.4.6 Field tests to investigate special effects (Annex IIIA 10.4.4)

B.9.4.7 Tunnel testing to investigate effects of feeding on contaminated honey (Annex IIIA 10.4.5)

Not required.

B.9.4.8 Exposure and risk assessment for bees (Annex IIIA 10.4)

First tier risk assessment for bees :

The risk assessment for bees is based on the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC of October 2002.

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil; the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is compressed with a roller. After a waiting period of 2 to 4 weeks, the soil is cultivated to allow the non-mineralised gasses to disperse. Since the product is phytotoxic, it is advisable to perform a germination test (Cress-test) on samples of soils collected from the treated field, before planting.

There is no direct spraying on plant material. The germination test proves that there is no concern of residues of metam-sodium or MITC in the planted crops. Therefore, bees are not at risk in-field and off-field since they are not exposed to contaminated crops or weeds. Bees are not exposed to the uses in greenhouse.

In conclusion, the risk of metam-sodium and its metabolite MITC to bees is acceptable for the intended uses.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; Annex IIIA 10.5)

There is no exposure of metam-sodium to foliage dwelling arthropods such as the two indicator species, *Typhlodromus pyri* and *Aphidius rhopalosiphii*. Only soil arthropods will be exposed to the soil fumigant. As a general soil fumigant, metam-sodium is known to have insecticidal properties. Clearly, the HQ based on the worst case scenario will be greater than or equal to the trigger value, indicating that there is a potential hazard to non-target arthropods. This leads to the conduct of higher tier tests.

Under these circumstances, the RMS can agree with the opinion of the notifier that the conduct of standard laboratory tests with the indicator species is irrelevant. Instead, the notifier provided 2 extended laboratory studies with rove beetles (*Aleochara bilineata*) and Collembola (*Folsomia candida*).

B.9.5.1 Effects of the active substance on non-target terrestrial arthropods (Annex IIA 8.3.2)

Studies are performed with the formulation Metam-Sodium 507 g a.s./L.

B.9.5.2 Effects of the formulations on non-target terrestrial arthropods (laboratory, semi-field tests) (Annex IIIA 10.5.1)

Extended laboratory study on the effects of Metam sodium on reproduction of Rove beetles *Aleochara bilineata* exposed to soil residues aged under field conditions. (Drexler *et al.*, 2002).

Guidelines :

Grimm *et al.* 2000: A test for evaluating the chronic effects of plant protection products on the rove Beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions.

SETAC (Barrett *et al.*, 1994)

GLP :

Yes

Material and Methods :

Test substance : Metam-Sodium, formulation containing 507 g a.s./L, batch n° : G 130013149

Test species : *Aleochara bilineata* (rove beetle)

Number of organisms, life stage, age : 4 replicates each with 10 males and 10 females, adults, 1-3 days old

Type of test : extended laboratory study

Test substrate :

Control and test item : natural soil from an arable field close to Rossdorf (Germany), silty sand to extremely loamy sand, 5.7 – 13.7 % clay, 31.1- 39.0 % silt, 51.4 – 57.4 % sand, 0.69 – 1.20 % organic carbon, < 0.1 % CaCO₃, mean soil pH : 5.18, maximum water capacity : 42.97 ± 1.90 %

Positive control : inert substrate, quartz sand : 0.4 – 0.8 mm particle size, 600 mL sand, moistened to 10 % (v/v) with deionised water (60 mL)

Applied concentrations :

control (400 L water/ha); positive control (1100 mL Perfekthion EC (417.5 g/L dimethoate) in 400 L water/ha) only used for the bioassay; test item (1 x 1200 L Metam-Sodium in 400 L water/ha, (equivalent to 608.4 kg a.s./ha))

The application of the test item was done on May 31, 2001. The application was done directly onto the soil surface according to agricultural practice. Immediately after application of each plot the test item was incorporated with a rotavator (depth of this rotavation treatment : approximately 15 – 25 cm) and the soil was compressed with a roller. The soil surface of the test item treated plots was re-opened on June 13, 2001. The test plots were mulched on June 30, 2001. *Phacelia* sp. was sown on July 07, 2001 to cover the soil surface.

The soil was aged under field conditions for 55 days. Soil samples were taken with a soil sampler of approximately 5 cm diameter up to 10 cm depth on July 25, 2001. From each of the 4 plots per treatment, 10 soil samples were taken from different locations (40 samples per treatment group) and were mixed afterwards. The soil was sieved to 5 mm. To minimize the influence of predators occurring in the soil, the samples were

defaunated. Therefore, the field soil was frozen for 2 days, kept at room temperature for 10 days and was frozen again for 3 days. The water amount of the soil was adjusted to about 35 ± 5 % of its maximum water holding capacity with deionised water.

Exposure route :

The exposure units for the control and the test item were plastic containers filled with soil collected in the field. Into each test unit, 10 pairs of beetles were introduced. Once a week *ca.* 500 *Delia antiqua* pupae per container were added on day 7, 14 and 21. The pupae were carefully buried into the soil (depth *ca.* 2-3 cm) and homogeneously distributed within the test unit and completely covered with the substrate.

The adult test organisms were exposed to the test item for 28 days. After 28 days, all surviving adult beetles were removed from the substrate and the number was recorded. The substrate and the parasitised onion fly pupae were returned to the climatic room in the original test units without a lid for one further week. 35 days after start of the bioassay, the pupae were washed out of the soil and the pupae of each replicate were transferred into a separate emergence container.

The parasitised fly pupae were placed in a funnel which was placed on a glass beaker; the bottom of the funnel is perforated with holes through which the hatched beetles fell into the glass beaker below. The pupae remained in the funnel. Emerging beetles of the new generation were counted and removed out of the emergence containers at least 3 times per week. Following 3 consecutive days of no beetle emergence, it was assumed that no further emergence would occur and the assessment was finished.

Test conditions :

temperature : 19 – 21 ° C (exposure), 20 – 22 ° C (post-exposure)

relative humidity : 65 – 85 % (exposure), 60 – 80 % (post-exposure)

photoperiod : 16/8 hours light/dark cycle

light intensity : 470 – 990 lux (exposure), 340 – 610 lux (post-exposure)

Findings :

Table B.9.5.2-1 : Effects of the formulation metam-sodium 507 g a.s./L on *Aleochara bilineata* in extended laboratory test

Evaluation criteria	Control	Positive control	Test item
Mean number of emerged beetles	636 ± 72	39 ± 21*	543 ± 20*
Reproduction relative to control (%)	-	- 93.9	- 14.6

* statistically significantly different from the control

No test item related behavioural abnormalities occurred.

Conclusions :

The study is acceptable.

The formulation Metam-Sodium 507 g a.s./L caused statistically significant, but only slight effects on reproduction capacity of rove beetles *Aleochara bilineata*, if applied at a rate up to 1200 L formulation/ha (equivalent to 608.4 kg a.s./ha) and aging of the soil under full field conditions for 55 days. Nevertheless, this effect is considered not to be biologically relevant as the reduction of reproduction compared to the control was only 14.6 %.

B.9.5.3 Effects of the formulations on non-target terrestrial arthropods (field tests) (Annex IIIA 10.5.2)

As a general soil fumigant, metam-sodium is known to have insecticidal properties. The risk of metam-sodium and its metabolite MITC should be addressed in terms of recovery in the field. An extended laboratory test is only able to assess the potential for recolonisation, but not actual recovery. Therefore, a semi-field or field study should demonstrate that the field can be recolonised with non-target arthropods from the border stripes (off-field). In this new study, several species should be tested (*Aleochara bilineata*, *Pardosa*, *Folsomia candida*, *Hypoaspis aculeifer*, ...).

Added in August 2010:

A field trial to determine the effects of METAM-SODIUM on the non-target arthropod fauna of arable land in SW-France after one application in spring. (Aldershof S., 2010).

Guidelines :

IOBC (Hassan, 1992), Anonymous (1992), ESCORT (Barrett *et al.*, 1994), Brown (1998) and IOBC, BART and EPPO Joint Initiative (Candolfi *et al.*, 2000)

GLP :

Yes (certified by Food and Consumer Product Safety Authority, The Netherlands)

Material and Methods :

Test substance : Nemasol 510, formulation containing nominally 510 g metam-sodium/L (analytically 499 g a.s./L), batch n°: G080311261

Test species : a natural non-target arthropod community in a commercial arable field

Type of test : field study

Test substrate: Poor sandy soil with low content of organic matter, on a commercial carrot field near Gabarret and Escalans, Les Landes, (France): 93.7 % sand ($0.05 < D < 2$ mm), 1.8 % coarse silt ($0.02 < D < 0.05$ mm), 1.8 % fine silt ($0.002 < D < 0.02$ mm), 2.7 % clay ($D < 0.002$ mm), 1.04 % organic matter, $< 3\%$ CO_3^{2-} , pH : 7.3

Test design : The trial had an unbalanced design with 4 replicates (plots of approximately 1 ha) for the water and test item treatments, and 2 replicates for the toxic reference item. Water and test item plots were alternated on 2 lines of 4 plots, the reference item plots were on the side.

Applied concentrations :

Control (1200 L water/ha); positive control (chlorpyrifos, equivalent to 5 kg a.i. in 300 L water/ha); test item (1 x 1200 L Metam sodium/ha, equivalent to 612 kg a.s./ha).

The test item was applied in accordance with GAP use, i.e. a single application at the rate mentioned. Due to time constraints applications took two days (7 and 8 April 2008). METAM-SODIUM was injected into the soil, immediately followed by sealing of the ground. Three weeks later the soil was ploughed in order to dispose of volatile chemical residues.

A water control treatment and a toxic reference treatment (chlorpyrifos) were run in parallel. The water control was applied by treating the soil in a similar way as the test item, but with water. In this way potential insecticidal properties of METAM-SODIUM could be directly compared to the control without the confounding effects of the application procedure. The toxic reference item chlorpyrifos was applied to the soil surface by overhead spray. This was done after the soil was treated with water in a way similar to the test item.

Aim of the test : The invertebrate taxa collected during treatment and at intervals thereafter were quantified – diversity and abundance – with different sampling methods in order to evaluate the immediate acute effects and the potential for recovery.

Exposure route:

Plots were situated in an area of approximately 12.5 ha. There was an overhead irrigation system, which dictated the semi-circular geometry of the field. Inside this field 10 plots were established. Water control and METAM-SODIUM treated plots measured approximately 1 ha. Reference Item plots were approximately 0.7 ha.

All plots were treated entirely. There were no untreated areas between the plots.

Sampling :

All samples were taken from the central 10 x 10 m area of each plot. Soil samples were taken on five random positions within the 10 x 10 m sampling area of each plot. Two groups of five pitfall traps were established in the central area of each plot, using two mirroring W-configurations. In the centre of this 10 by 10 m area, one photo-elector and one pan trap were placed. The positions of the photo-elector traps were changed after each sample. All traps were removed and reinstalled for ploughing, harvesting the carrots, and sowing of the maize (using GPS information)

Sampling methods: soil sampling, pitfall trap sampling, photo-elector sampling and pan trap sampling

- soil sampling : Small soil inhabiting arthropods, e.g. mite families and Collembola, were sampled from the upper 5 cm soil layer with a core drill.
- pitfall trap sampling : Soil and surface dwelling arthropods, e.g. carabids and spiders, were sampled using pitfall traps. The traps consisted of a plastic funnel buried flush with the soil surface. A collection jar containing ethanol 70% was tightly attached to the funnel
- photo-elector sampling : To assess the abundance of soil dwellers and to monitor the emergence of arthropod prey items such as thrips from the soil, photo-elector traps were set up. The traps were circular with a ground surface of 25 cm² and a total height of 95 cm. The collection container was filled with 70% ethanol.
- pan trap sampling : The purpose is to assess the abundance and biodiversity of highly mobile taxa, to reassure that pitfall trap catches included all taxa prevailing in the study area. In addition, pan trap data can provide background information about food availability and migration of some taxa during certain periods. The in-crop flight activity of insects was monitored with yellow pan traps placed within the canopy. The pans were filled with water and some detergent or ethylene-glycol.

Sampling occasions : Samples were collected throughout the trial period from 2 April 2008 until 21 May 2009. Population samples were taken before application (two samples end of March/early April); 10 days after application (mid April); 4 weeks after application (between ploughing and sowing: mid May), and 6, 9, 12, 18, 23 and 29 weeks after application. Additional pitfall and soil samples were taken late November, and in spring the following year (mid March, mid April and end of May 2009). Usually a soil sample, pitfall sample, photo-elector sample and pan trap sample were taken at or around the same day.

Collected organisms : In total 333 thousand arthropods were identified: 67% were collected from pitfalls, 19% from soil samples, 11% from pan trap samples and 2% from photo electors. When data became available, it appeared that pan trapping and photo-elector sampling methods were inappropriate for evaluation of potential differences in local population dynamics on this experimental scale and in this habitat type. Evaluations are therefore exclusively based on soil sample data and pitfall data.

- Community analysis: 102 taxa were included: 83 taxa from pitfall- and 19 taxa from soil samples
- Population analysis: 46 taxa with a high abundance (at least 10 and 15 individuals in respectively pitfall and soil samples of the water control on several sampling occasions)

Test conditions: average meteorological conditions

Statistics :

Effects on ground dwelling arthropod populations were examined using ordination techniques applied to the entire dataset. Effects of the test item treatment at the community level were expressed relative to the control using the Principal Response Curve method. At the population level abundant taxa were examined statistically using ANOVA or the non-parametric Mann Whitney-U test.

Test performance :

The results presented in this report show that the test system was sufficiently sensitive, and that application and soil- and pitfall sampling methods were adequate to detect statistically significant effects at an α level of 0.1 or 0.05 in case they occurred. In the toxic reference treatment almost 80% (soil samples) and 50% (pitfall samples) of all taxa examined at the population level were adversely affected.

A thorough survey of the entire arthropod community prevailing in the soil and on the soil surface of the carrot field was established using these two sampling methods. Clear qualitatively different responses could be detected. Sampling provided a comprehensive dataset, which allowed for a valid and robust evaluation of potential treatment effects at the community level and at the population level.

Findings :

1 – Soil samples

Community response

Soil injection with METAM-SODIUM lead to an abrupt adverse effect on the soil inhabiting mite- and Collembola community (Figure B.9.5.3-1A). The overall community response was statistically significantly different from the control at an alpha level of 0.05 (Table B.9.5.3-1). On individual sampling dates differences compared to the control were statistically significant on almost all post-application sampling occasions in 2008, except on the last occasion on 20 November, although the response curve had not entirely reached the zero-line at that time. Sampling in 2009 confirmed that recovery processes had led to full recovery of the entire mite- and small arthropod community in May.

When analysed in more detail, part of the community had a maximum response immediately after soil treatment, followed by a gradual recovery. From October onwards, canonical coefficients were close to zero, indicating that full recovery of this arthropod assemblage was established well within one year after treatment (Figure B.9.5.3-2A; PRC 1+2). The species weights diagram indicated that Scutacaridae largely contributed to this response pattern (upper right quadrant of Figure B.9.5.3-1B).

Another part of the community showed a gradual build-up of the response. The maximum canonical coefficient was observed in August. Recovery started in September, but the response curve did not entirely reach the zero-line. The oribatid mite Oppiidae had the largest influence on this community response pattern (lower right quadrant of Figure B.9.5.3-1B).

Single taxa

Densities of all taxa collected from soil core samples that were present during application were initially reduced after injection with METAM-SODIUM (Table B.9.5.3-2). Only Nanorchestidae appeared indifferent to the soil treatment with the test item. Populations disappeared after injection both in the water control treatment and in the test item treatment, and re-appeared in both treatments in July/August, at similar densities. For several taxa recovery was observed within approximately one or two months after treatment (Eupodidae, Pygmephoridae, Tarsonemidae, Acaridae, Histiostomatidae). The majority of taxa remained adversely affected during the entire season, but in autumn densities were similar or almost similar to the control (Gamasida, Scutacaridae, Tydeidae, oribatid taxa and Arthropleona). Populations of Gamasida and Oribatida were again reduced compared to the control by more than 50% in spring the next year. On the last sampling occasion in May 2009 differences compared to the control were no longer statistically significant, and had reduced to ecologically acceptable levels.

Enchytraeidae numbers found in soil samples were too low for statistical analysis, but dynamics did not indicate adverse treatment related effects.

Table B.9.5.3-1 : Summary performance PRC analyses

treatments included in analysis	% Variance accounted for by		% Variance explained by treatment regime captured by		P-value ax1	P-value ax2
	time	treatment	ax1	ax2		
all	48.6	19.9	35.5	20.7	0.015	0.001
T1	52	14.5	57.2	12.0	0.026	0.197
R	49.5	17.6	47.2	17.3	0.070	0.132

P-values at individual sampling moments (Monte Carlo Permutation Test)

date	sample	T1	Reference
02-Apr-08	1	0.255	0.216
06-Apr-08	2	0.593	0.199
17-Apr-08	3	0.027	0.062
03-May-08	4	0.047	0.064
18-May-08	5	0.041	0.075
12-Jun-08	6	0.048	0.075
02-Jul-08	7	0.106	0.063
08-Aug-08	8	0.065	0.066
17-Sep-08	9	0.068	0.053
20-Oct-08	10	0.019	0.068
20-Nov-08	11	0.577	0.120
17-Mar-09	1	0.185	0.481
10-Apr-09	2	0.523	0.135
21-May-09	3	0.061	0.092

Soil sample data

treatments included in analysis	% Variance accounted for by		% Variance explained by treatment regime captured by		P-value ax1	P-value ax2
	time	treatment	ax1	ax2		
all	66.8	11.8	31.4	10	0.012	0.009
T1	70.3	5.9	22.1	15.6	0.036	0.136
R	69.1	10.7	37.6	15.8	0.069	0.062

P-values at individual sampling moments (Monte Carlo Permutation Test)

date	sample	T1	Reference
30-Mar-08	1	0.255	0.216
05-Apr-08	2	0.593	0.199
17-Apr-08	3	0.027	0.062
03-May-08	4	0.047	0.064
18-May-08	5	0.041	0.075
12-Jun-08	6	0.048	0.075
02-Jul-08	7	0.106	0.063
08-Aug-08	8	0.065	0.066
16-Sep-08	9	0.068	0.053
28-Oct-08	10	0.019	0.068
21-Mar-09	11	0.157	0.127
14-Apr-09	12	0.062	0.203

Pitfall sample data

all All treatments analyzed together
T1 Test item treatment analyzed separately
Reference Reference item analyzed separately

Statistically significant at alpha = 0.1
Statistically significant at alpha = 0.05

T1 METAM-SODIUM
Reference chlorpyrifos

The reference item has lower statistical power due to lower replication (2 vs 4 test item replicates)

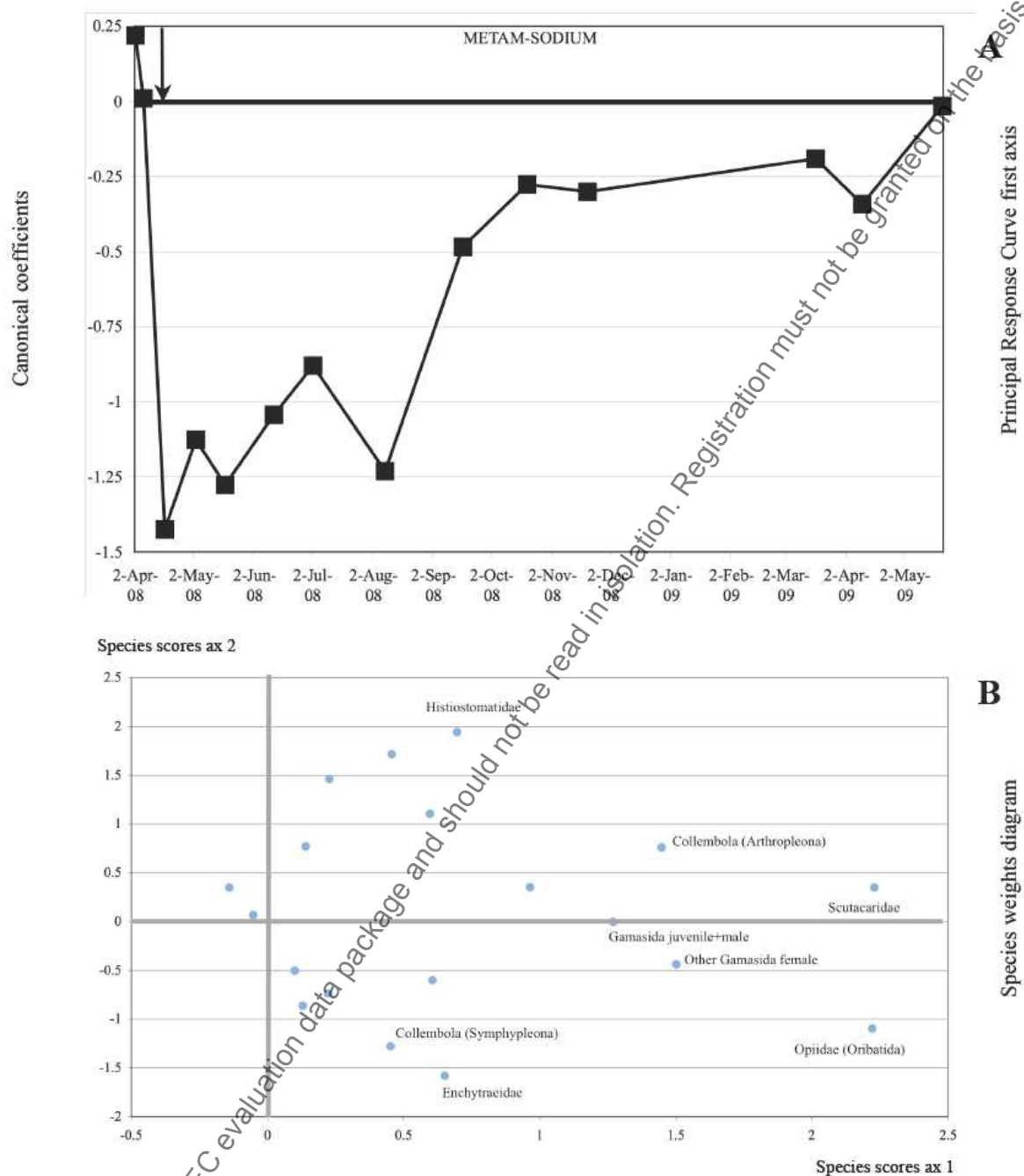


Figure B.9.5.3-1 : Community response test item treatment PRC 1 (soil sample data)

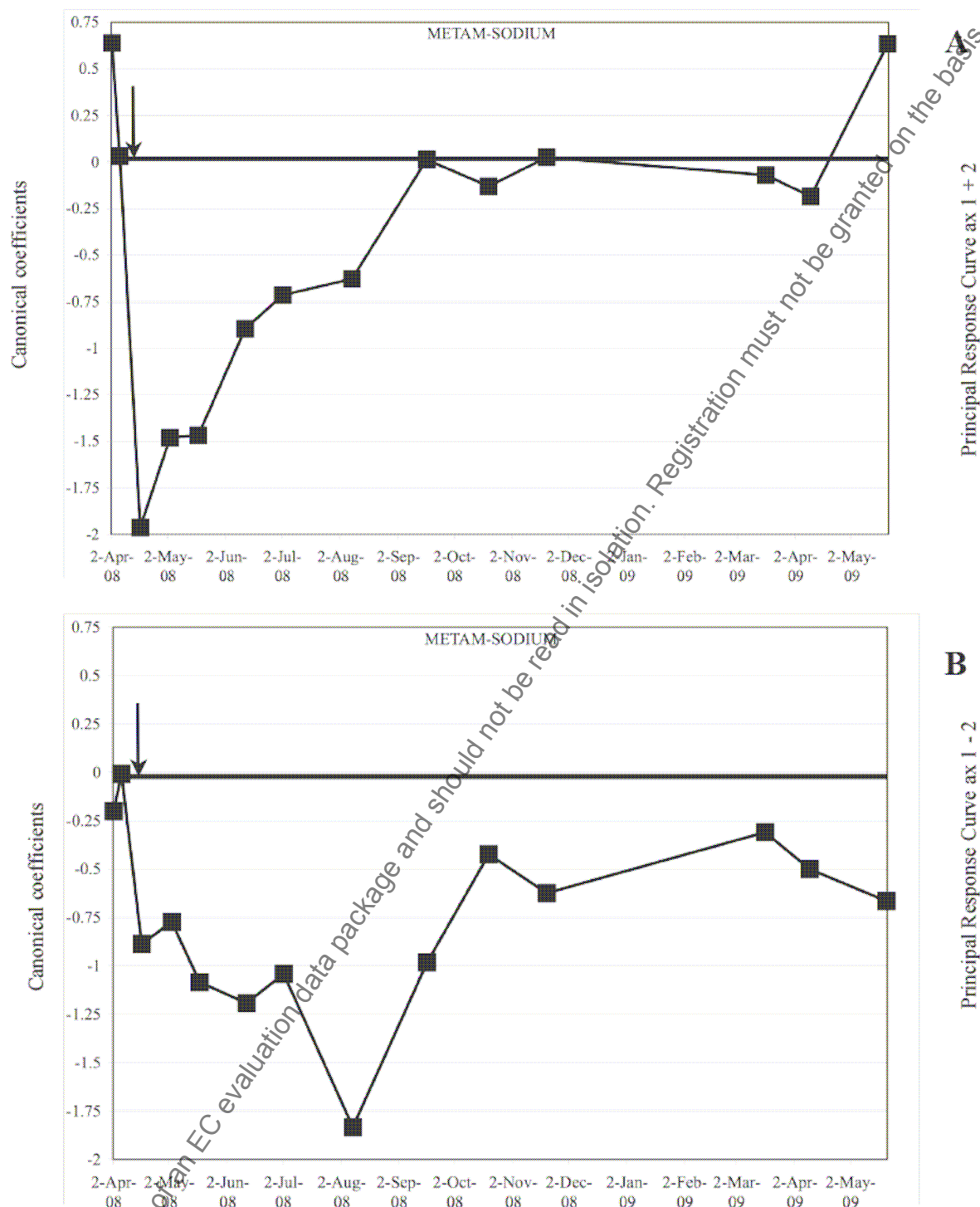


Figure B.9.5.3-2 : Community response test item treatment PRC 1 and 2 (soil sample data)

Table B.9.5.3-2 : Percentage reduction and statistical significances soil samples

	2008										2009				
	sample date	1 2-Apr	2 6-Apr	3 17-Apr	4 3-May	5 18-May	6 12-Jun	7 2-Jul	8 8-Aug	9 17-Sep	10 20-Oct	11 20-Nov	1 17-Mar	2 10-Apr	3 2-May
Test Item															
Gamasida female		2.6%	6.4%	89.5%	90.1%	58.1%	53.1%	55.0%	93.0%	64.6%	45.9%	26.9%	35.6%	72.9%	40.0%
Gamasida juvenile+male		-21.0%	0.0%	91.2%	90.8%	28.0%	18.2%	80.0%	71.0%	63.3%	1.6%	36.7%	38.7%	49.2%	8.1%
Gamasida total		-7.1%	5.3%	90.2%	90.4%	43.9%	40.4%	68.3%	67.1%	63.4%	26.4%	32.4%	36.0%	61.1%	33.2%
Eupodidae		52.4%								90.0%	59.1%	32.5%	25.0%	46.4%	
Nanorchestidae										-50.0%	-64.7%	-58.9%	-36.3%		-35.1%
Pygmephoridae		-68.7%	-25.0%			100.0%			32.9%	-15.4%	-21.6%	-79.7%	29.3%	-50.0%	
Scutacaridae		-20.8%	-5.1%	90.5%	93.9%	94.6%	98.1%	100.0%	88.7%	14.8%	-11.5%	-52.0%	29.3%	-30.2%	-65.4%
Tarsonemidae		-53.6%	47.2%	64.4%	70.7%	4.5%	15.4%	79.7%			-30.3%		-66.3%	-17.4%	-69.7%
Tydeidae								93.0%	96.4%	48.1%	-45.3%	81.3%	-58.9%		
Acaridae		-42.6%	-18.5%	68.6%	69.2%	22.5%	-54.1%	21.5%	63.7%	11.3%	70.3%	33.5%	-48.0%	-13.2%	-19.9%
Histiogmatidae		-76.1%		94.0%	92.3%	6.3%									
Opilidae (Oribatida)		-66.1%	-29.0%	86.2%	92.0%	80.9%	75.1%	89.8%	90.3%	74.0%	47.5%	43.0%	44.9%	79.1%	50.0%
Oribatida other		47.3%	42.0%	66.0%	78.1%	90.2%	81.4%		-36.0%	-32.4%	63.3%	66.3%	57.3%	61.7%	12.1%
Collembola (Arthropleona)		-46.0%	-6.7%	71.3%	73.3%	96.0%	88.1%	16.2%	77.5%	16.5%	27.3%	39.6%	-16.1%	-25.0%	-61.2%
Collembola (Symphypleona)		55.3%								27.3%		65.1%	79.2%	21.4%	
Reference															
Gamasida female		1.3%	-31.0%	81.1%	71.8%	-3.1%	50.0%	80.0%	92.4%	81.1%	-15.9%	30.8%	61.3%	50.8%	80.9%
Gamasida juvenile+male		50.0%	-39.7%	64.7%	56.9%	4.0%	45.5%	10.0%	73.9%	63.8%	8.2%	34.7%	57.3%	72.3%	51.6%
Gamasida total		25.5%	-33.8%	77.3%	63.2%	1.8%	50.9%	46.3%	87.5%	55.8%	-5.4%	35.2%	59.3%	59.6%	70.7%
Eupodidae		81.0%								100.0%	77.3%	66.3%	50.0%	92.9%	
Nanorchestidae										46.2%	43.7%	-4.4%	-68.2%		50.0%
Pygmephoridae		-18.4%	33.3%			-38.1%			94.5%	-40.4%	-48.2%	-4.8%	82.1%	39.1%	
Scutacaridae		-60.4%	-3.3%	35.6%	39.4%	32.0%	67.3%	75.0%	51.8%	-65.4%	-54.8%	-86.5%	8.5%	-50.0%	-61.9%
Tarsonemidae		22.9%	31.7%	59.6%	75.6%	0.0%	38.5%	69.5%			-23.3%		-26.4%	32.4%	-81.1%
Tydeidae								-61.6%	-18.6%	84.4%	40.4%	96.3%	59.0%		
Acaridae		-6.7%	23.0%	96.9%	90.7%	97.4%	99.3%	100.0%	96.5%	21.1%	67.4%	76.6%	5.5%	42.4%	80.3%
Histiogmatidae		-40.0%		100.0%	100.0%	100.0%									
Opilidae (Oribatida)		19.4%	53.1%	-37.9%	85.7%	48.9%	82.8%	83.7%	95.0%	-8.3%	46.2%	-2.9%	-49.4%	-3.4%	-38.1%
Oribatida other		-15.7%	6.8%	-28.4%	43.8%	41.5%	62.8%		-11.1%	-84.8%	3.1%	-51.9%	52.2%	40.4%	-51.7%
Collembola (Arthropleona)		-42.8%	-18.4%	86.0%	76.5%	94.1%	89.9%	44.1%	93.9%	1.8%	33.2%	47.6%	77.5%	35.4%	66.2%
Collembola (Symphypleona)		39.5%								100.0%		69.6%	91.7%	78.6%	

64% Abbott effect above 50%

-54% Abbott effect below -50%

P-value <0.05

P-value <0.1

ANOVA without blocking factor/Dunnett's test two sided, or non-parametric (Mann-Whitney U)
if error variances are not homogeneous (Levene's test)

If total number in water control treatment is below 15 (total of all replicates), no Abbott values are calculated

2 – Pitfall samples

Community response

Initially the overall PRC showed only a mild adverse effect of METAM-SODIUM treatment to the above ground arthropod community. The overall response pattern expressed by the first PRC axis showed a gradual build-up of adverse effects from July onwards, with a maximum amplitude at the end of the year (Figure B.9.5.3-3A). In spring 2009 canonical coefficients were closer to zero, but still not entirely back to the control response. The overall community response obtained from the pitfall dataset was statistically significant at an alpha level of 0.05 (Table B.9.5.3-1).

When incorporating the second PRC axis, two highly distinct community patterns were obtained. One pattern showed an initial positive effect, turning slowly into an increasing negative response at the end of the season. Next spring the adverse effect had reduced, but was still markedly visible (Figure B.9.5.3-4A, PRC 1+2). The other pattern displayed an immediate adverse effect. Recovery was initiated quickly, but full recovery occurred only the following year (Figure B.9.5.3-4B, PRC 1-2).

On individual sampling occasions statistically significant differences ($\alpha=0.05$) compared to the control were seen on four consecutive sampling occasions immediately after application, and on the last sampling occasion that was analysed in 2008. Differences were not statistically significant at this α -level in analysed samples of 2009 (Table B.9.5.3-1). It is indicated by the species weights diagram (Figure B.9.5.3-3A) that Heteroptera contributed largely to the delayed response pattern (Figure B.9.5.3-4A; PRC 1+2), and that the immediate response pattern (Figure B.9.5.3-4B; PRC 1-2) was much influenced by Oribatida.

Single taxa

- Coleoptera

Carabid taxa responded differentially towards soil injection treatment with METAM-SODIUM (Table B.9.5.3-3). *Amara* showed no adverse effects. *Harpalus* was initially unaffected, but densities were moderately reduced at the end of the year and in spring the following season. On the sampling occasion in April 2009 effects were below 50% and no longer statistically significant (Table B.9.5.3-3). Other carabids, mainly containing *Calathus* and *Nebria*, were similarly affected, i.e. statistically significant reductions were observed only in autumn (Table B.9.5.3-3). In spring 2009 densities were comparable to the control again.

Cucujoidean families, including juvenile and adult Coccinellidae, were not harmed by METAM-SODIUM soil injection. Histeridae, mainly present in spring, did not show any adverse test item treatment related effects either. In spring the following year densities were also similar to the control.

The staphylinid genera Aleocharinae and Omaliinae were unaffected by the test item treatment. Staphylininae species on the other hand were moderately reduced compared to the control on several occasions during the entire post-application sampling period in 2008 (Table B.9.5.3-3). In spring 2009, densities were comparable to the control, confirming full recovery. Xantholininae populations appeared reduced in October 2008, but not during other periods (Table B.9.5.3-3). Other Staphylinidae did not show consistent treatment related effects.

Juvenile Coleoptera collected in pitfall traps were reduced in numbers compared to the control immediately after the soil injection treatment. In autumn numbers were reduced again, but differences compared to the control were not statistically significant (Table B.9.5.3-3). Other Coleoptera were also moderately reduced at the end of the year 2008 (Table B.9.5.3-3). At that time mainly Pselaphidae occurred. Adverse treatment effects were no longer visible in 2009.

- Araneae

Most spider populations developed similarly in control plots and METAM-SODIUM treated plots. Only Gnaphosidae showed a clearly treatment related reduction in numbers in the METAM-SODIUM plots from June onwards (Table B.9.5.3-3). In spring 2009 densities were generally low, but populations appeared to have recovered in the test item treatment. Lycosidae other than *Pardosa* showed a transient reduction shortly after METAM-SODIUM treatment, but differences compared to the control were not statistically significant (Table B.9.5.3-3).

- Other arthropods

Hemipteran taxa were not affected by the test item treatment (Table B.9.5.3-3), but the number of true bugs (Heteroptera) was lower in METAM-SODIUM plots throughout the entire sampling period from June onwards. Heteroptera collected in the carrot field were mainly Miridae, Cydnidae and Lygaeidae. In spring 2009 populations were still moderately reduced, but differences compared to the control were no longer statistically significant (Table B.9.5.3-3).

Nematocera were unaffected by soil treatment with METAM-SODIUM. Orthorrhapha showed a transient adverse effect in June and July, whereas Acalyptratae were moderately reduced in November 2008 and March 2009. Differences compared to the control were not statistically significant (Table B.9.5.3-3). Higher Diptera (Calyptrata) were again unaffected. Juvenile Diptera on the other hand were statistically significantly reduced immediately after METAM-SODIUM treatment in spring 2008 (Table B.9.5.3-3).

The hymenopteran taxa Apoidea and Formicidae showed no adverse treatment related effects. Chalcid wasps (mainly Mymaridae) were reduced in METAM-SODIUM treated plots later during the season, which may indicate an indirect effect through their host. Specimens were not identified to the genus level. In spring 2009 densities were similar to the control. In one water plot an exceptionally high number of Mymaridae were collected in April 2009, otherwise densities were very similar in all treatment plots. Scelionidae numbers were statistically significantly reduced compared to the control up to two months after METAM-SODIUM treatment, but not later during the season (Table B.9.5.3-3). Populations of other Proctotrupoidea (mostly Diapriidae) were approximately 80% reduced up to two months after application, and again later during the season (Table B.9.5.3-3). These population dynamics suggest a direct effect followed by a delayed indirect effect through their host. In spring 2009 densities were low, but populations appeared recovered.

Collembolan populations sampled with pitfalls showed a transient but statistically significant reduction up to two months after application. Treatment effects on collembolans sampled from the soil layer (pitfalls) were less persistent than effects deduced from populations living within the soil (soil core samples).

Plant inhabiting Orthoptera and Thysanoptera had population developments which were very similar in all treatment plots. Ground dwelling Diplopoda, Chilopoda and Oribatida were obviously affected by the METAM-SODIUM treatment. Diplopoda populations recovered after approximately two months. Chilopoda and Oribatida numbers remained lower for a longer period, but recovery was observed before the end of the year in both taxa. Sampling in 2009 confirmed that population dynamics were similar to the control again (Table B.9.5.3-3).

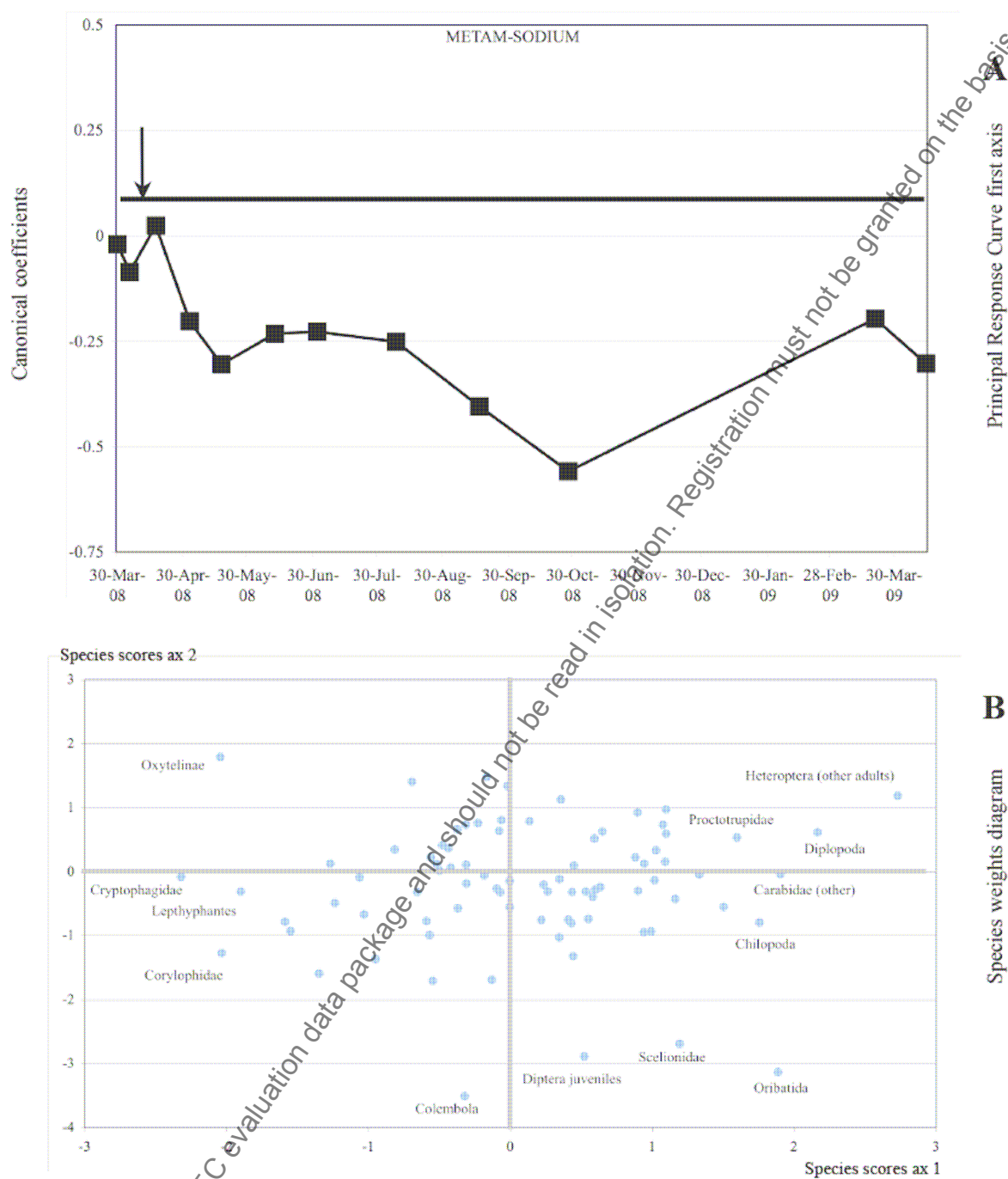


Figure B.9.5.3-3 : Community response test item treatment PRC 1 (pitfall sample data)

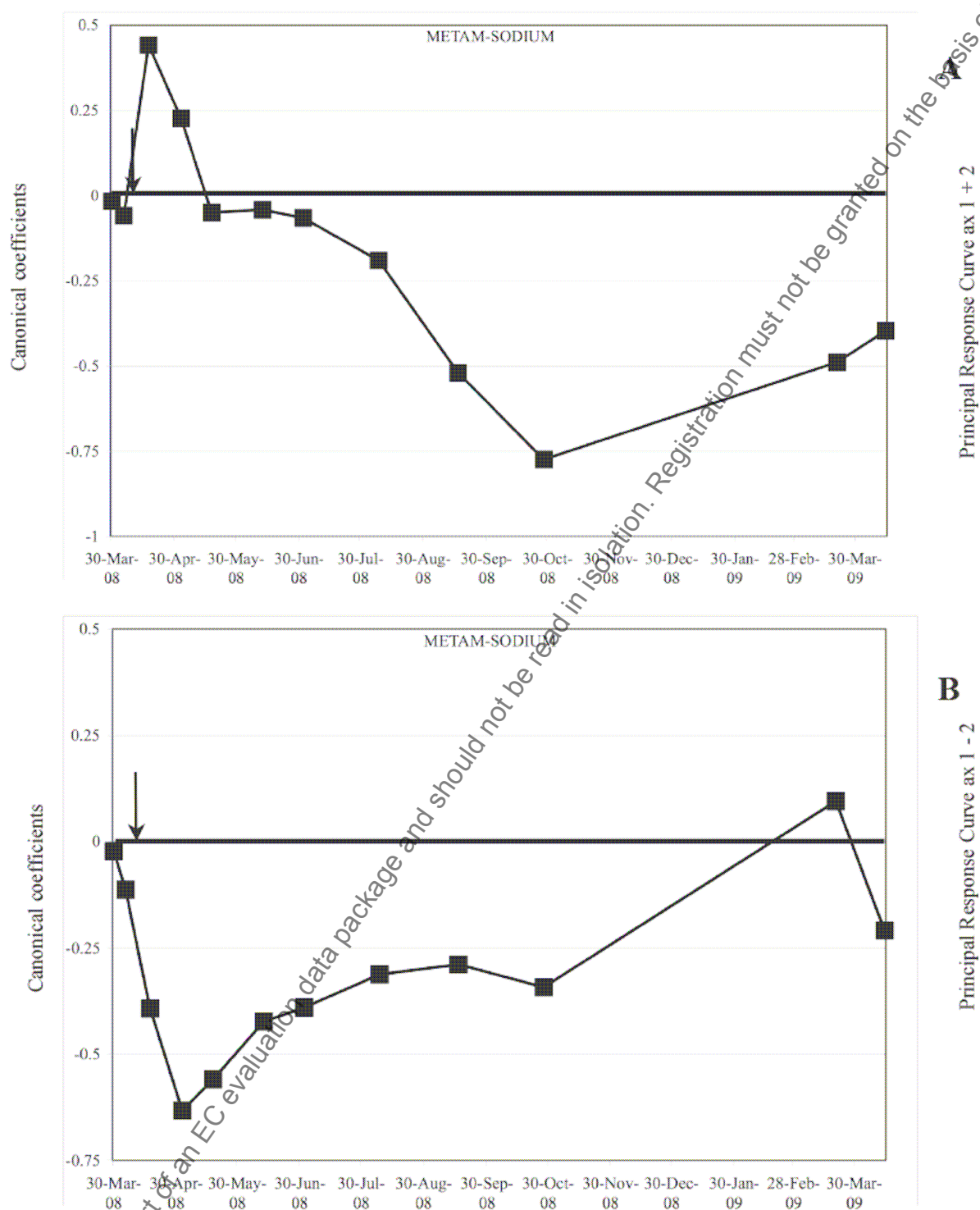


Figure B.9.5.34: Community response test item treatment PRC 1 and 2 (pitfall sample data)

Table B.9.5.3-3 : Percentage reduction and statistical significances METAM-SODIUM pitfall samples

	2008										2009	
	1 30-Mar	2 5-Apr	3 17-Apr	4 3-May	5 18-May	6 12-Jun	7 2-Jul	8 8-Aug	9 16-Sep	10 28-Oct	R1 21-Mar	R2 14-Apr
Collembola	-6.9%	13.9%	79.6%	78.4%	60.5%	12.1%	4.0%	-40.5%	-39.2%	-30.5%	-59.1%	-14.4%
Orthoptera							10.0%	19.7%	56.8%	32.2%		
Thysanoptera	9.1%	53.1%	-51.4%	1.6%	29.7%	37.5%	16.8%	29.7%	-15.5%	-12.5%	-46.4%	10.8%
Aphidoidea	-66.4%	4.9%	-26.1%	0.0%		31.1%	-57.0%	-45.0%	-65.8%	-60.0%	-56.0%	63.6%
Cicadellidae	64.3%						-38.1%	20.9%	57.1%	45.1%	30.4%	-8.0%
Delphacidae	60.0%							-10.3%	3.9%	-60.1%	-38.2%	75.1%
Heteroptera		75.0%	-47.8%	-20.0%	37.5%	-17.4%	8.3%	60.6%	56.4%	76.2%	63.7%	60.0%
Sciaridae	-14.6%	4.5%	7.1%	-22.5%	23.0%	-55.2%	-46.4%		7.0%	-22.9%	9.2%	-32.9%
Nematocera (other)	47.2%	53.5%	9.5%	-31.3%	50.0%			-53.3%	74.3%			-24.2%
Orthorrhapha					20.0%	29.4%	63.2%	50.0%	-38.6%			
Acalyptratae	20.4%	27.8%	-32.8%	2.2%	-57.5%	30.3%	-63.9%	-68.6%	-3.1%	56.1%	52.0%	-14.0%
Calyptratae	8.8%		-69.0%	10.8%	-11.6%	-47.8%	13.6%	0.0%	63.3%			-3.9%
Diptera (juveniles)	10.0%		90.3%	75.0%	79.2%			-33.3%	53.8%			-32.9%
Amara spp.	19.3%	-11.8%	5.2%	-55.5%	25.0%						-13.7%	23.5%
Harpalus spp.	-60.8%	19.5%	3.5%	32.3%	16.3%	15.6%	-19.9%	-47.6%	30.1%	43.1%	64.0%	48.8%
Carabidae (other)	25.4%	16.0%	17.9%	41.0%	38.6%	47.1%	22.9%	17.3%	76.4%	66.6%	-13.6%	-44.4%
Coccinellidae (juveniles)							-19.4%					
Coccinellidae (adults)						16.7%		50.8%				
Cryptophagidae	66.7%		33.3%	-51.9%		-30.0%	0.0%	-33.6%				-68.8%
Histeridae			21.4%	63.9%	20.0%	28.2%						
Lathridiidae	-44.9%	-34.7%	-42.5%	-67.4%	-64.7%	8.3%	53.5%	-43.9%	39.3%	-27.6%	14.5%	-41.9%
Cucujoidae other		39.5%	50.0%	-8.5%		-4.3%	56.9%	9.0%			8.7%	
Aleocharinae	-2.9%	8.9%	6.3%	-22.8%	48.2%	-30.0%	-1.3%	9.8%	-25.5%	-16.9%	12.9%	6.4%
Omalinae	-30.3%	0.4%	50.0%	0.0%	23.1%	63.3%	-25.6%	-57.8%	57.1%	-26.9%	34.7%	
Staphylininae	-5.9%	9.9%	63.6%	37.5%	52.9%		-13.6%	36.8%	56.5%	61.4%		48.8%
Xantholininae	-17.3%	-8.9%	25.3%	29.0%	49.4%	81.1%	56.4%			88.2%	34.8%	18.8%
Staphylinidae (other)	15.5%	-26.0%	61.1%				83.3%	-5.6%			35.0%	-31.3%
Coleoptera (other juveniles)	-2.5%	-27.9%	65.0%	80.7%	33.0%	-40.0%	46.7%	-76.7%	-17.3%	55.6%	30.8%	-25.0%
Coleoptera (other adults)	-2.2%	0.8%	-27.8%	17.8%	49.2%	-14.3%	-15.4%	-23.6%	57.8%	65.8%	28.8%	22.4%
Apoidea				5.7%		20.0%						
Formicidae			-54.4%	-42.9%	63.6%	-38.2%	28.3%		17.6%		46.5%	58.6%
Chalcidoidea								50.0%	59.3%	62.9%		
Scelionidae	-53.1%	17.5%	38.0%	85.2%	62.7%	44.1%	27.6%	21.7%	6.1%	-20.0%	-48.0%	-3.2%
Proctotrupoidea (other)	-59.4%		13.0%	84.6%					14.1%	79.0%		
Diplopoda	-9.3%	37.6%	-90.8%	23.3%	72.6%	69.4%	81.0%	46.8%	-25.2%	-39.5%	-31.1%	32.6%
Chilopoda							85.7%		74.8%	0.0%		
Oribatida	27.7%	21.7%	72.6%	82.6%	70.3%	73.0%	79.1%	6.3%	33.0%	38.1%	-22.0%	37.3%
Phalangida (juveniles)	-30.1%	27.0%	40.0%		-27.8%	43.5%	72.6%	36.2%	3.1%	-46.9%	-59.4%	
Phalangida (adults)					0.0%	29.9%	11.4%	-0.4%	-6.0%	-1.2%		
Gnaphosidae								70.0%	56.5%	55.6%		
Pardosa	-1.2%	2.1%	26.5%	-29.6%		40.0%	-2.1%	19.1%	14.5%	20.5%	-14.5%	-29.6%
Lycosidae other	32.4%	-16.6%	52.0%	26.1%	16.7%	-20.8%	20.0%	18.2%	1.6%	-11.9%	20.6%	3.1%
Pachygnatha	-14.7%	15.1%	-25.3%					-62.5%	-32.3%	-46.7%	-26.1%	-28.3%
Oedothorax apicatus	-10.2%	41.8%	35.6%	53.8%	-23.5%	-1.3%	2.8%	-47.5%	-55.4%	-34.7%	-5.9%	-47.7%
Erigoninae (other)	-22.0%	3.9%	-33.3%	20.0%	-8.3%	47.8%	-44.4%	16.2%	-41.0%	-40.5%	-4.6%	-43.4%
Linyphiinae (other)			25.9%		-10.5%	-8.7%	31.7%	42.3%	-20.4%		1.0%	

64% Abbott effect above 50% -54% Abbott effect below -50%

□ P-value <0.05

□ P-value <0.1

ANOVA without blocking factor/Dunnett's t-test (two sided) or Mann-Whitney U if error variances are not homogeneous (Levene's test)

If total number in water control treatment is below 10 (total of all replicates), no Abbott values are given

Table B.9.5.3-4 : Overview population effects METAM-SODIUM

TAXON	method	effect	significant	Total period of effect	Recovery 1	Recovery 2	
ACARI							
Gamasida females	soil	+	+	entire season	Sep-08	Oct-08	R
Gamasida males+juveniles	soil	+	+	entire season	Aug-08	Sep-08	R
Eupodidae	soil	+	+	ca. 2 months	Oct-08	Nov-08	
Nanorchestidae	soil	-	-				
Pygmephoridae	soil	+	-	ca. 2 months	Jun-08	Jun-08	
Scutacaridae	soil	+	+	entire season	Aug-08	Sep-08	
Tarsonemidae	soil	+	+	<1 month	May-08	May-08	
Tydeidae	soil	+	+	entire season	Sep-08	Sep-08	
Acaridae	soil	+	-	<1 month	May-08	May-08	
Histiogmatidae	soil	+	+	<1 month	May-08	May-08	
Opiidae	soil	+	+	entire season	Jul-08	Oct-08	R
Oribatida other	soil	+	+	entire season	Jul-08	Aug-08	R
OTHER							
Collembola Arthropleona	soil	+	+	entire season	Jun-08	Sep-08	
Collembola Symphypleona	soil	+	-	entire season	Mar-09	Mar-09	R
COLEOPTERA							
Amara spp.	pitfall	-	-				
Harpalus spp.	pitfall	+	+	<1 month	Mar-09	Mar-09	R
Carabidae (other)	pitfall	+	+	ca. 2 months	Mar-09	Mar-09	R
Coccinellidae (juveniles)	pitfall	-	-				
Coccinellidae (adults)	pitfall	-	-				
Cryptophagidae	pitfall	-	-				
Lathridiidae	pitfall	-	-				
Cucujoidea other	pitfall	-	-				
Histeridae	pitfall	-	-				
Aleocharinae	pitfall	-	-				
Omalinae	pitfall	-	-				
Staphylininae	pitfall	+	+	entire season	Jun-08	Mar-09	
Xantholininae	pitfall	+	+	ca. 2 months	Mar-09	Mar-09	R
Staphylinidae (other)	pitfall	-	-				
Coleoptera (other juveniles)	pitfall	+	+	<1 month	May-08	May-08	
Coleoptera (other adults)	pitfall	+	+	ca. 2 months	Mar-09	Mar-09	
ARANEAE							
Phalangida (juveniles)	pitfall	-	-				
Phalangida (adults)	pitfall	-	-				
Gnaphosidae	pitfall	-	-				
Pardosa	pitfall	+	+	entire season	Sep-08	Mar-09	
Lycosidae other	pitfall	+	-	<1 month	Apr-08	Apr-08	
Pachygnatha	pitfall	-	-				
Oedothorax apicatus	pitfall	-	-				
Erigoninae (other)	pitfall	-	-				
Linyphiinae (other)	pitfall	-	-				
DIPTERA							
Sciaridae	pitfall	-	-				
Nematocera (other)	pitfall	-	-				
Orthorrhapha	pitfall	+	-	ca. 2 months	Sep-08	Sep-08	R
Acalyptratae	pitfall	+	-	ca. 2 months	Apr-09	Apr-09	R
Calypttratae	pitfall	-	-				
Diptera (juveniles)	pitfall	+	+	ca. 2 months	Aug-08	Aug-08	
HYMENOPTERA							
Apoidea	pitfall	-	-				
Formicidae	pitfall	-	-				
Chalcidoidea	pitfall	+	-	entire season	Mar-09	Mar-09	R
Scelionidae	pitfall	+	+	ca. 2 months	Jun-08	Jun-08	
Proctotrupoidea (other)	pitfall	+	+	entire season	Jul-08	Apr-09	R
OTHER							
Collembola total	pitfall	+	+	ca. 2 months	May-08	Jun-08	
Orthoptera	pitfall	-	-				
Thysanoptera	pitfall	-	-				
Aphidoidea	pitfall	-	-				
Cicadellidae	pitfall	-	-				
Delphacidae	pitfall	-	-				
Heteroptera	pitfall	+	+	>1 year	spring-09	not observed	
Diplopoda	pitfall	+	+	ca. 2 months	Jul-08	Aug-08	
Chilopoda	pitfall	+	+	entire season	Oct-08		
Oribatida	pitfall	+	+	entire season	Aug-08	Aug-08	

Significant: $P=0.1$

+/: yes; -: no

Recovery 1: rate of increase similar to the control

Recovery 2: effect values below 50% and not statistically significant.

R1 Populations appeared recovered at the end of the year, but in spring populations were reduced compared to the control again. However, on the last sampling moment reductions were below 50% and not statistically significant.

R2 Initially, populations did not appear to be adversely affected. Only later in the season or the next spring effects were detected.

3 – Overview evaluations

Soil samples

Small arthropods living in the upper soil layer were immediately and severely affected by METAM-SODIUM soil injection. This was confirmed by community analyses (Figure B.9.5.3-1) as well as population level evaluations (e.g. Table B.9.5.3-2). More than 80% of the taxa extracted from soil samples (mites and Collembola) were adversely affected during any moment of the study period. Half of all soil inhabiting populations were reduced for a period longer than two months (Figure B.9.5.3-5). At the sampling occasion immediately following METAM-SODIUM soil injection 80% of the taxa examined at the population level was adversely and statistically significantly reduced compared to the control (Figure B.9.5.3-6). This percentage fluctuated between 20% and 60% up to four months after application. On subsequent sampling occasions in 2008 the percentage of adverse and statistically significantly affected taxa remained below the 10% threshold level. In April 2009 this figure went up once to 25%, but on the last sampling occasion (21 May 2009) no statistically significant adverse effects were detected (Figure B.9.5.3-6).

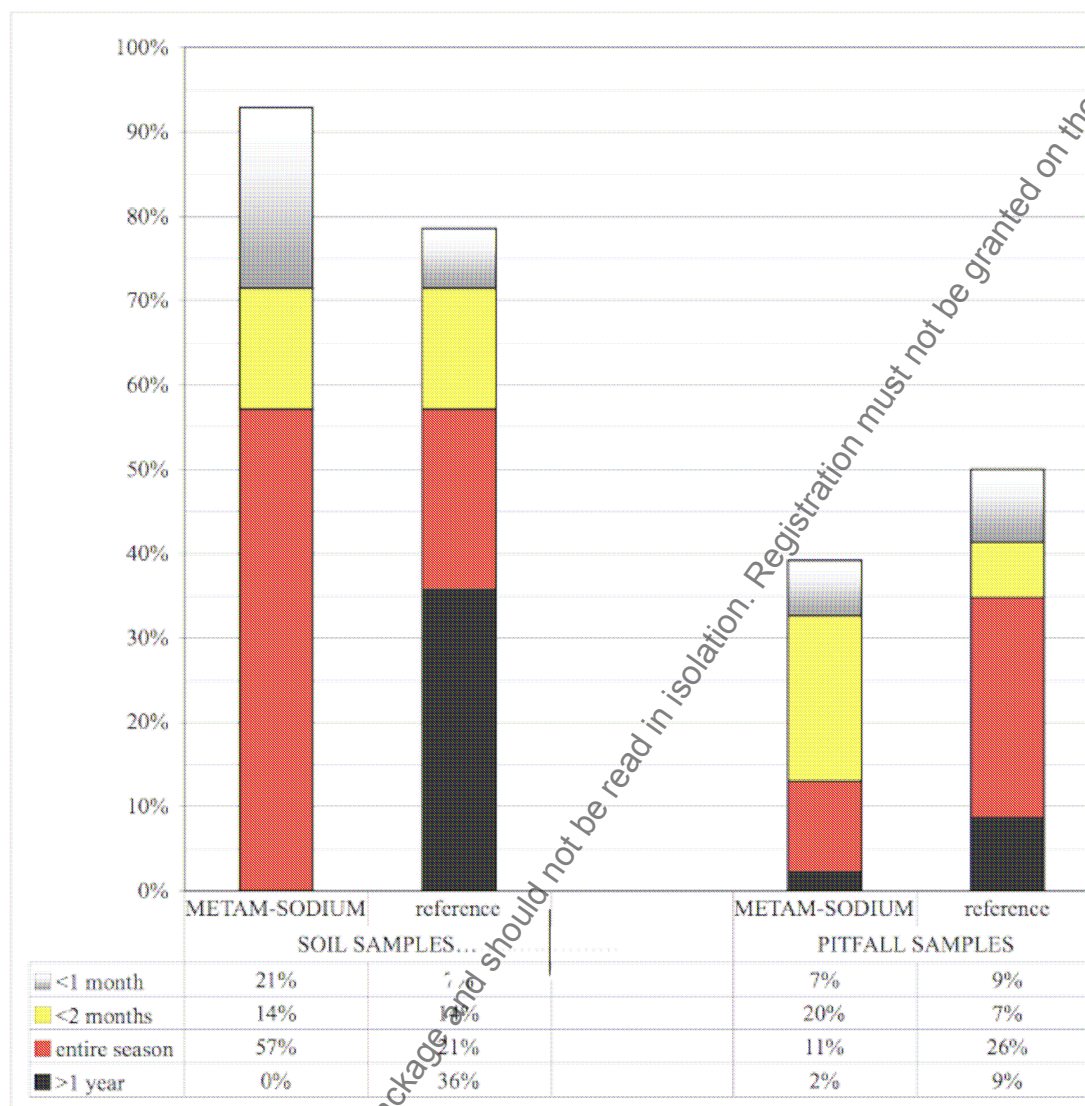
The community response displayed by the first ordination axis (Figure B.9.5.3-1) very much resembles the significant response curve (Figure B.9.5.3-6), representing the proportion of adverse and statistically significantly affected taxa over time ($\alpha=0.1$).

In comparison to the reference item (overspray with chlorpyrifos) METAM-SODIUM induced adverse effects to a larger number of taxa prevailing in the upper soil layer (Figure B.9.5.3-5). However, at the end of the sampling period all taxa had recovered in METAM-SODIUM treated plots, whereas this was not the case in toxic reference treated plots. This also showed in the significant response curve graph (Figure B.9.5.3-6), despite the low replication in the reference item treatment (two replicates vs. four replicates in the control and the test item treatment).

Pitfall samples

METAM-SODIUM soil injection induced a rather different response pattern to the above ground arthropod community, sampled with pitfalls (Figure B.9.5.3-3). Effects were less abrupt and more moderate. Less than 40% of the taxa examined at the population level were adversely affected at any time point during the course of the study (Figure B.9.5.3-5). At individual sampling occasions at maximum 20% of the taxa were adversely and statistically significantly affected. This was in May, five weeks after application (Figure B.9.5.3-7). Community analyses detected a gradual build-up of a second adverse response. This additional delayed response was also detected in the significant response curve, albeit less clearly (Figure B.9.5.3-7). It is hypothesized that these delayed effects could be due to initial effects on juvenile stages emerging from the ground (Heteroptera, Carabidae, Staphylinidae, Diptera), or through adverse effects on hosts (Mymaridae, Proctotrupoidea other than Scelionidae). Several juvenile stages of arthropods were collected infrequently in pitfall samples (possibly because of lower mobility, or because they did not live on the soil surface in that particular life stage). Soil samples were appropriate for abundant arthropods smaller than 500 μm but not for larger specimens. Hence potential adverse effects on soil inhabiting macroarthropods did not appear immediately. However, when more adult stages of the new generation were collected in pitfalls later during the season, adverse effects gradually increased. Because indirect effects usually show more variation, these effects are less often statistically significant and were therefore less evident in the significant response curve (Figure B.9.5.3-7). The variation was however detected in community analyses (Figure B.9.5.3-3A or Figure B.9.5.3-4A), which is known to be a very sensitive method (Smit *et al.*, 2002).

Direct overspray with the reference item induced strong adverse and immediate responses to 50% of all taxa examined (Figure B.9.5.3-5 and Figure B.9.5.3-7). The above ground arthropod fauna was affected more by the toxic reference overspray than by METAM-SODIUM soil injection.



Test item: METAM-SODIUM soil injection
Reference item: chlorpyrifos overspray soil application

Interpretation of adverse effects in this figure is based on graphical examination of consistent trends and Abbott effects (>50%). Taxa that were not statistically significantly reduced compared to the control but showed nonetheless a consistent pattern indicating adverse treatment related effects are included in these counts.

Figure B.9.5.3-5 : Percentage of adversely affected arthropod taxa

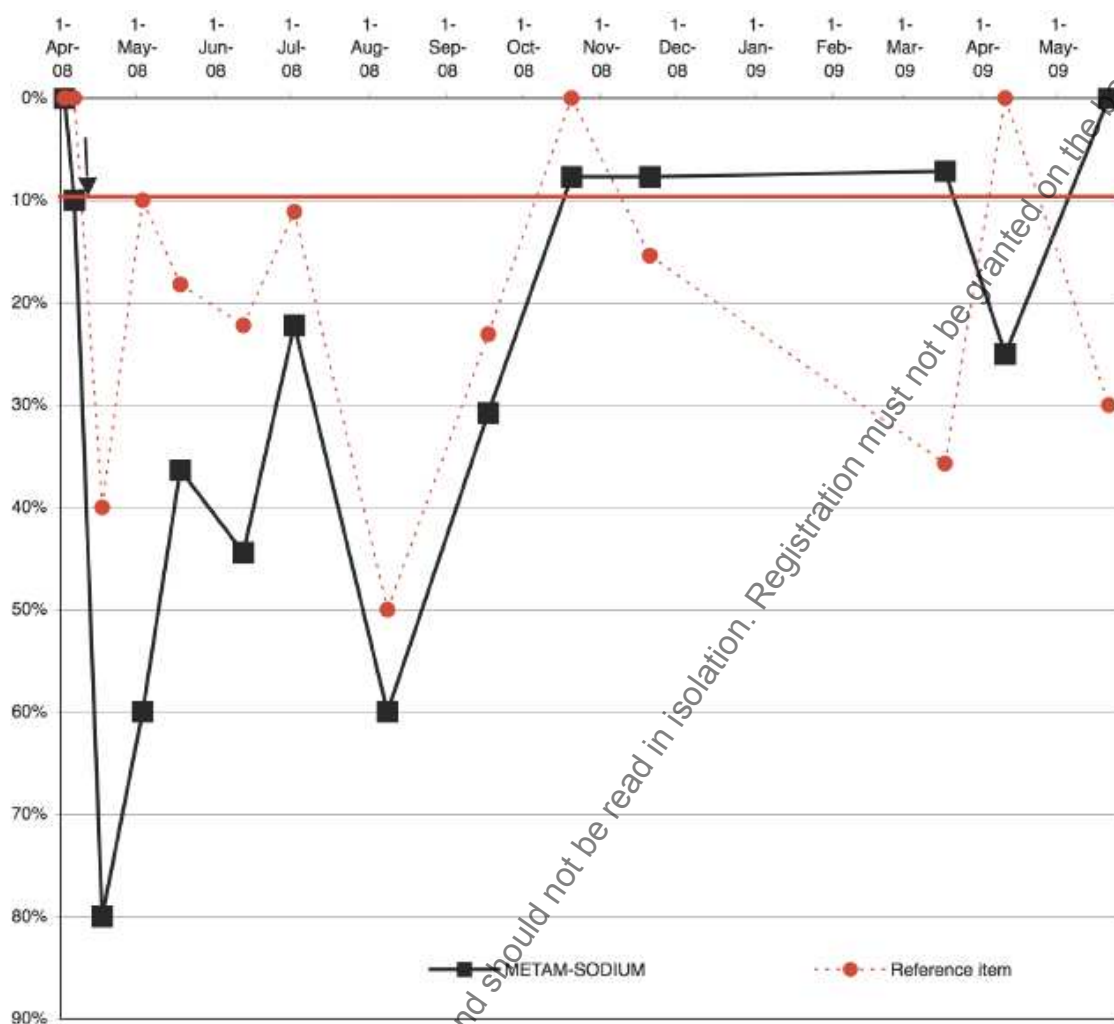


Figure B.9.5.3-6 : Significant response curves (soil sample data)

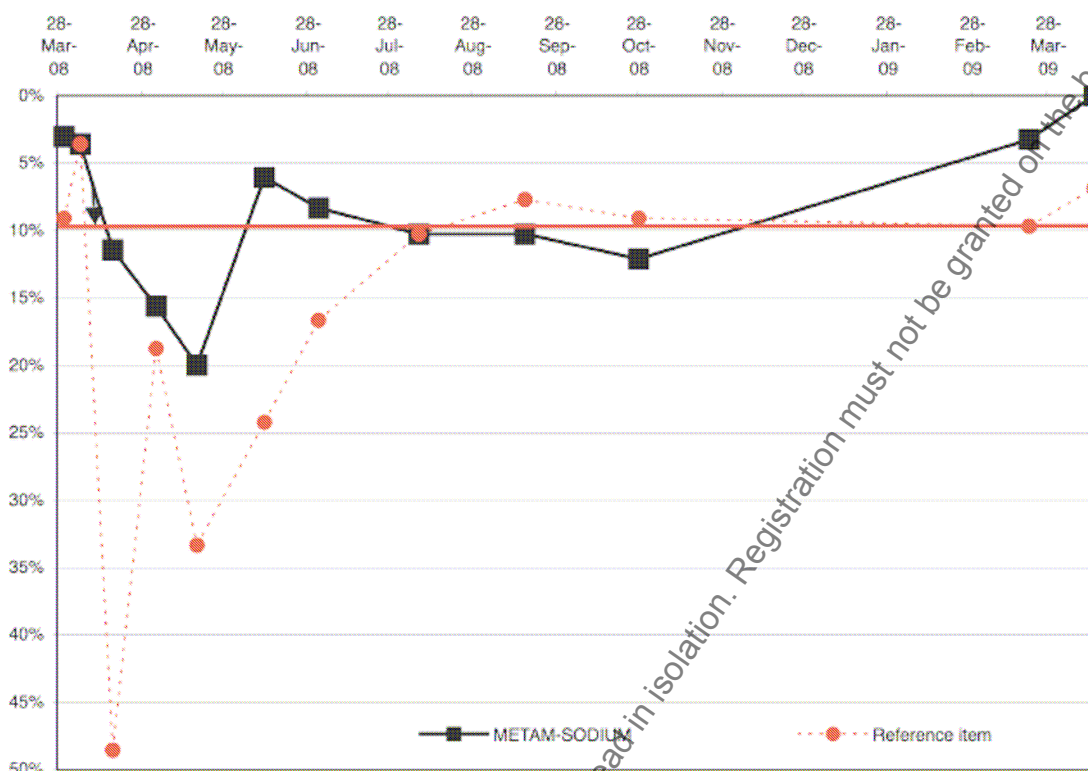


Figure B.9.5.3-7 : Significant response curves (pitfall sample data)

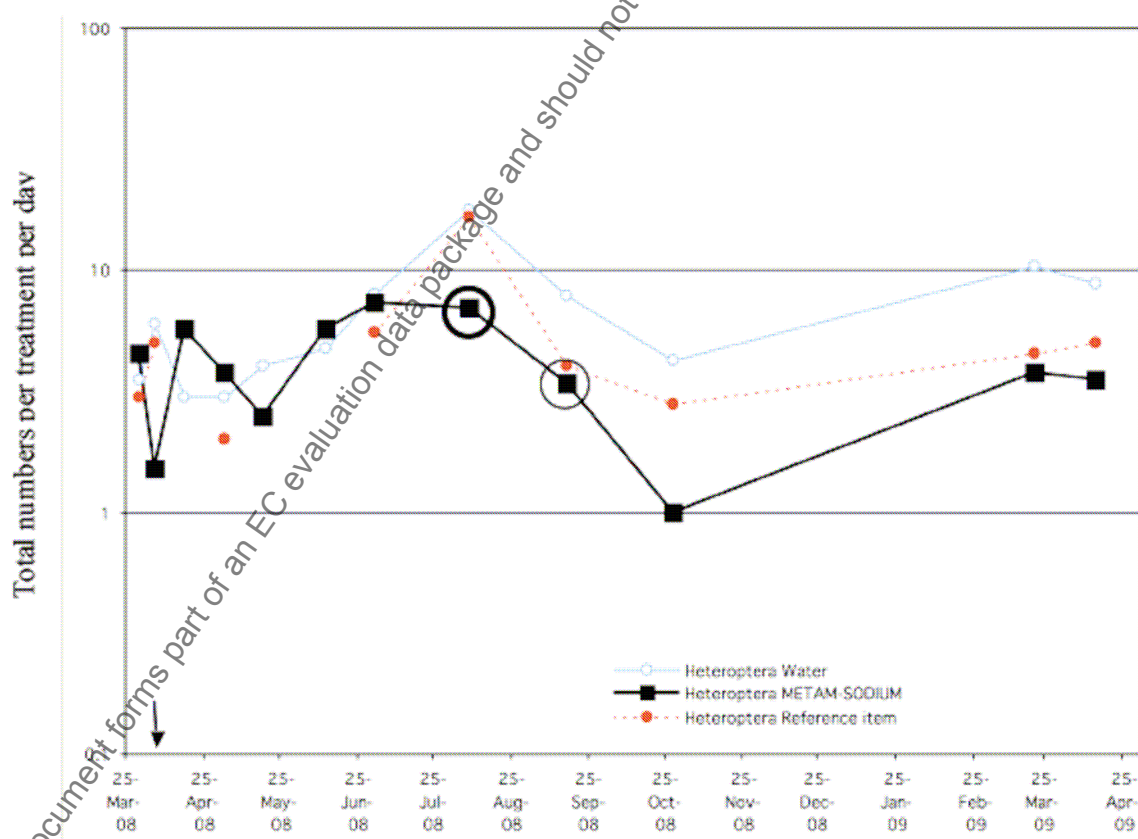


Figure B.9.5.3-8 : Density graphs Heteroptera (pitfall samples)

Conclusions :

-A wide array of arthropod taxa was sampled from the upper soil layer and from the soil surface. The reference item treatment showed that the test set-up was sufficiently sensitive, and that qualitatively different responses could be detected in case these occurred.

-The community of soil inhabiting small arthropods (mite taxa and springtails) were markedly and persistently affected by METAM-SODIUM soil injection at a rate of 612 kg a.s./ha. More than 90% of all taxa examined at the population level were adversely affected. In autumn most populations appeared to have recovered. In early spring, at the onset of population development, some populations were reduced again compared to the control (Gamasida, Oppiidae and other Oribatida), but on the last sampling occasion reductions had diminished to ecologically acceptable levels and differences compared to the control were no longer statistically significant (see also B.9.7).

-The community of above ground soil dwelling larger arthropods were moderately and transiently affected by METAM-SODIUM soil injection at a rate of 612 kg a.s./ha. Less than 40% of all taxa examined at the population level were adversely affected. At the end of the season a second delayed adverse response was observed, which could have been induced through initial adverse effects on juvenile arthropods living in the soil during application, or through adverse direct effects on parasitoid hosts. The following spring population development of predominant taxa was similar to the control. Heteroptera was still moderately reduced in the next season but numbers were generally low and differences compared to the control were not statistically significant (see Figure B.9.5.3-8).

The soil injection of Metam-sodium at a rate of 612 kg a.s./ha has an initial adverse effect on both the soil inhabiting small arthropods and the above ground dwelling larger arthropods.

After application, a gradual recovery was observed. During the following autumn for the soil inhabiting small arthropods or at the beginning of next spring for the above ground dwelling larger arthropods, no significant differences were observed in the treated field compared to the controls.

Conclusions of the RMS :

The study is acceptable. The field study is well performed (different taxa were sampled with soil and pitfall sampling) and good reported (evaluations by different statistical methods).

Metam-sodium application at the highest rate of 612 kg a.s./ha has initial adverse effects on soil arthropods living in the soil and at the soil surface. The field study demonstrates that actual recovery in the field will occur for the most important taxa within one year:

- For the soil samples 0% taxa showed a recovery > 1 year. The arthropod taxa that would be expected to be most at risk from a soil injected treatment such as metam-sodium would be the small and relatively immobile soil dwelling taxa such as Collembola, soil mites, larvae of soil dwelling beetles (e.g. Aleocharinae and some Carabidae). All of these groups showed clear recovery within one year of application of metam-sodium (see also B.9.7).
- For the pitfall samples only 2% taxa showed a recovery > 1 year. From Table B.9.5.3-4 it appears that of all the sampled taxa the only taxon that did not show full recovery within one year was Heteroptera. Also from Figure B.9.5.3-3A (PRC 1+2) it is clear that Heteroptera contributed largely to the delayed response pattern. Heteroptera are primarily foliage dwelling arthropods and therefore not strongly associated with bare soil, which is the GAP for the application of metam. From Figure B.9.5.3-8 it is clear that any effects of metam-sodium on Heteroptera did not begin to occur until 4 months after treatment. Such a long delay to onset of effects is due to an indirect effect, either the decline in numbers of a suitable prey (for example aphids) on the carrot plants or decline in quality of a host plant (for phytophagous Heteroptera). The numbers of Heteroptera in metam-sodium treated plots were not statistically different from controls in samples taken the year after treatment. Despite being reported as not having shown recovery, the data shows that no difference was observed in absolute numbers just before and one year after treatment.

For the most important soil inhabiting and soil surface dwelling species the recovery occurs within one year. For 2% of pitfall sampling taxa recovery is still ongoing in the next spring. Therefore, RMS proposes that potential risk mitigation measures are taken into consideration at the Member State level.

B.9.5.4 Summary of effects, exposure and risk assessment for non-target terrestrial arthropods

Revised in August 2010:

Table B.9.5.4-1 : Summary of effects of metam-sodium on non-target arthropods : Extended laboratory study

Species	Life stage	Test substance, substrate and duration	Dose (kg a.s./ha) ^{1,2}	End point	% effect ³	Trigger value
<i>Aleochara bilineata</i>	adults	Metam-Sodium 507 g a.s./L, aged field soil, 28 d	608.4 kg a.s./ha, aged for 55 days	Reproduction	- 14.6 %	50 %

¹ indicate whether initial or aged residues

² for preparations indicate whether dose is expressed in units of a.s. or preparation

³ indicate if positive percentages relate to adverse effects or not

effect on reproduction negative values : adverse effects; positive values : no adverse effects

First tier risk assessment for non-target arthropods :

The risk assessment for non-target arthropods is based on the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC of October 2002.

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil; the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is compressed with a roller. After a waiting period of 2 to 4 weeks, the soil is cultivated to allow the non-mineralised gasses to disperse. Since the product is phytotoxic, it is advisable to perform a germination test (Cress-test) on samples of soils collected from the treated field, before planting. There is no direct spraying on plant material. The germination test proves that there is no concern of residues of metam-sodium or MITC in the planted crops. Therefore, leaf-dwelling arthropods are not at risk in-field and off-field since they are not exposed to contaminated crops or weeds.

Non-target arthropods are not exposed to the uses in greenhouse.

The effects of the treatment with the formulated Metam-Sodium were tested on the soil-dwelling arthropod *Aleochara bilineata*. A bioassay was performed with treated soil after 55 days of aging under field conditions. No biologically relevant effects were observed for the reproduction at the application rate of 1 x 1200 L Metam-Sodium/ha (608.4 kg a.s./ha). This aged residue study demonstrates that *Aleochara bilineata* is able to recolonise the field after 55 days of aging.

However, the risk of metam-sodium and its metabolite MITC should be addressed in terms of recovery in the field. An extended laboratory study is only able to assess the potential for recolonisation, but not actual recovery. Therefore, a semi-field or field study should demonstrate that the field can be recolonised with non-target arthropods from the border stripes (off-field). In this new study, several species should be tested (*Aleochara bilineata*, *Pardosa*, *Folsomia candida*, *Hypoaspis aculeifer*, ...).

A field trial (Aldershof S., 2010) was performed to determine the effects of metam-sodium on the non-target arthropod fauna of arable land in SW-France after one application in spring. The field study is well performed (different taxa were sampled with soil and pitfall sampling) and good reported (evaluations by different statistical methods).

Metam-sodium application at the highest rate of 612 kg a.s./ha has initial adverse effects on soil arthropods living in the soil and at the soil surface. The field study demonstrates that actual recovery in the field will occur for the most important taxa within one year:

- For the soil samples 0% taxa showed a recovery > 1 year. The arthropod taxa that would be expected to be most at risk from a soil injected treatment such as metam-sodium would be the small and relatively immobile soil dwelling taxa such as Collembola, soil mites, larvae of soil dwelling beetles (e.g. Aleocharinae and some Carabidae). All of these groups showed clear recovery within one year of application of metam-sodium (see also B.9.7).
- For the pitfall samples only 2% taxa showed a recovery > 1 year. From Table B.9.5.3-4 it appears that of all the sampled taxa the only taxon that did not show full recovery within one year was Heteroptera. Also from Figure B.9.5.3-3A (PRC 1+2) it is clear that Heteroptera contributed largely to the delayed response pattern. Heteroptera are primarily foliage dwelling arthropods and therefore not strongly associated with bare soil, which is the GAP for the application of metam. From Figure B.9.5.3-8 it is clear that any effects of metam-sodium on Heteroptera did not begin to occur until 4 months after treatment. Such a long delay to onset of effects is due to an indirect effect, either the decline in numbers of a suitable prey (for example aphids) on the carrot plants or decline in quality of a host plant (for phytophagous Heteroptera). The numbers of Heteroptera in metam-sodium treated plots were not statistically different from controls in samples taken the year after treatment. Despite being reported as not having shown recovery, the data shows that no difference was observed in absolute numbers just before and one year after treatment.

For the most important soil inhabiting and soil surface dwelling species the recovery occurs within one year. For 2% of pitfall sampling taxa recovery is still ongoing in the next spring. In conclusion, the risk of metam-sodium and its metabolite MITC to non-target arthropods is sufficiently addressed for the field uses at the EU level. RMS proposes that potential risk mitigation measures are taken into consideration at the Member State level.

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

B.9.6.1 Acute toxicity to earthworms (Annex IIA 8.4.1)

B.9.6.2 Sublethal effects on earthworms (Annex IIA 8.4.2)

B.9.6.3 Acute toxicity of the formulations to earthworms (Annex IIIA 10.6.1.1)

B.9.6.4 Sublethal effects of the formulation on earthworms (Annex IIIA 10.6.1.2)

Not required. An earthworm field study was performed with the formulation Metam-Sodium 507 g a.s./L.

B.9.6.5 Field tests – residue content of earthworms (Annex IIIA 10.6.1.3)

Field study to evaluate the effects of Metam sodium on earthworms. (Lühns U., 2002).

Guidelines :

Federal Biological Research Centre for Agriculture and Forestry (BBA) F.R.G., June 1994 : Guideline for testing pesticides for registration, part VI, Nr. 2-3 : Effects of plant protection products to earthworms in the field.

ISO/CD Guideline 11268-3 (1997) : Soil quality – effects of pollutants on earthworms. Part 3 : Guidance on the determination of effects in field situations.

GLP :

Yes

Material and Methods :

Test substance : Metam-Sodium, formulation containing 507 g a.s./L, batch n° : G 130013149

Test species : naturally occurring field population of :

epilobous : *Aporrectodea caliginosa*, *Aporrectodea limicola*, *Allolobophora chlorotica*, *Aporrectodea rosea*, *Octolasion tyraeum*, *Octolasion cyaneum*
tanylobous : *Lumbricus castaneus*, *Lumbricus terrestris*

Life stages, sex, age : juveniles and adults, hermaphrodites, according to natural phenology

Type of test : field test

Test site : The study was carried out on an arable field test site, situated in Darmstadt-Dieburg, Rossdorf in Germany. The size of the test area was ca. 5000 m². In 1998 winter wheat and in 1999 winter barley was cultivated. In 2000, the field was uncultivated after harvest of winter barley.

Test design : The experiment was of randomized block design with 5 treatments (untreated control, agricultural control, positive control, treatment with 300 L Metam-Sodium/ha and treatment with 1200 L Metam-Sodium/ha), each with 4 replicates. Each plot measured 100 m² (12.5 m x 8 m) with 3 m distance between the plots in the rows and 7 m distance between the plots in the columns.

Soil profile of the field : loamy sand to silty sand

5.7 – 13.7 % clay, 33.1 – 39.0 % silt, 51.4 – 57.4 % sand, 0.69 – 1.20 % organic carbon, < 0.1 % CaCO₃

Treatments : (May 31, 2001)

- untreated control
- agricultural control (to check the mechanical impact)
- positive control : 8 kg Du Pont Benlate (50 % benomyl) in 400 L water/ha (equivalent to 4 kg a.s./ha)
- single treatment at 300 L Metam-Sodium/ha (152.1 kg a.s./ha)
- single treatment at 1200 L Metam-Sodium/ha (608.4 kg a.s./ha)

One week before application (May 24, 2001), a few weeds were cut and left in the field. The application of Metam-Sodium was on bare soil on May 31, 2001. After application, each plot was immediately treated with a rotavator to incorporate the test item up to a depth of 25 cm and compressed with a roller. Two weeks after the application (June 13, 2001), the soil was re-opened with a cultivator. Another soil cultivation was performed on July 7, 2001 using a rotary harrow to a depth of 5-8 cm. During this last cultivation, 10 kg/ha *Phacelia tanacetifolia* was sown using a seed drill.

In the agricultural control the same soil cultivation was performed. The positive control and the untreated control were not cultivated.

During the growing season, the plant cover in the Metam-Sodium treated groups and the agricultural control was more or less equal. In winter 2001/2002, *Phacelia tanacetifolia* was killed by frost (in every plot) and the soil was only covered by the remains of the dead *Phacelia tanacetifolia* and the wild weeds. Since the density of wild weeds in the test item groups (and especially in the 1200 L/ha group) was lower than in the agricultural control, the test site did not ensure exactly the same conditions for every treatment group until the fourth sampling, though in spring 2002 fallen off *Phacelia tanacetifolia* seeds were growing again. This means that in winter 2001/2002 between the third and the fourth sampling the plant cover in the test item group (especially in the 1200 L/ha group) was lower than in the controls. The reduced plant cover provides a lower source of food and is therefore representing worst case conditions.

Exposure time : approximately one year (from May 31, 2001 until May 22, 2002)

Earthworm sampling collection dates :

1st collection (pre-sampling) : May 7 and 8, 2001

2nd collection (3^{1/2} weeks after treatment) : June 25 and 26, 2001

3rd collection (4^{1/2} months after treatment) : October 15 and 16, 2001

4th collection (1 year after treatment) : May 21 and 22, 2002

4 samples were collected from all plots at each sampling collection date. The sample area was 0.25 m² for each sample (1m² for each plot/sample date). Only the area within the plots was used for sampling. Each of the 4 samples was collected at a distance of at least 2 m from each other. The sampling was done with the electric octet-method developed by Thielemann (1986). The method efficacy was proved by digging out (depth 30-50 cm) prior sampled areas and checking the soil by hand for remaining earthworms.

Earthworm determination : Species of all adult earthworms were determined using the glass-tube method for living earthworms (Thielemann, 1986). Species of juvenile earthworms were determined if possible, but were at least separated in tanylobous and epilobous worms. The determination was done according to Graf (1953) and Sims and Gerard (1985) within the shortest possible time (up to 1 day) after sampling.

Earthworm biomass : Collected adults were individually weighted. Collected juveniles were weighted in groups of tanylobous and epilobous for each sample.

Test conditions :

1st earthworm collection : air temperature : 11.0 – 21.2 °C, soil temperature : 10.9 – 13.2 °C

application date, May 31, 2001 : air temperature : 20.3 – 29.2 °C
wind-velocity : 0.1 m/s (up to 5.4 m/s for short periods in which application stopped)
relative humidity : 26.4 – 50.0 %
soil temperature : no value available

2nd earthworm collection : air temperature : 24.5 – 29.6 °C, soil temperature : 18.4 – 21.3 °C

3rd earthworm collection : air temperature : 12.4 – 21.7 °C, soil temperature : 13.4 – 14.2 °C

4th earthworm collection : air temperature : 19.6 – 27.3 °C, soil temperature : 15.2 – 16.7 °C

Table B.9.6.5-1 : Mean values of water content, Maximal Waterholding Capacity and pH of the soil

Earthworm sampling date	pH	Water content (%)	Maximum Waterholding capacity (%)
1 st collection date (May 7, 2001)	5.1	18.8	47.86
2 nd collection date (June 25+26, 2001)	5.2	15.6	not determined
3 rd collection date (October 15, 2001)	5.0	20.9	not determined
4 th collection date (May 21+22, 2002)	4.9	15.3	not determined

Findings :

The study is considered valid.

The mean value of the first earthworm collection was 79.2 % individuals/m² (mean over all plots) at study initiation and thus was higher than 20 earthworms/m².

Important species of the 1st collection :

Aporrectodea caliginosa : 70.9 % of determined species

Allolobophora chlorotica : 12.4 % of determined species

Lumbricus castaneus : 14.2 % of determined species

and thus were 3 species with more than 10 %.

Mean efficacy of the earthworm sampling method :

1st collection : 83.1 % (74.2 – 88.9 %)

2nd collection : 65.7 % (41.2 – 80.0 %)

3rd collection : 84.2 % (74.4 – 88.2 %)

4th collection : 71.1 % (63.0 – 85.2 %)

and thus was higher than 60 %.

The decrease of the earthworms abundance of the positive control at the 2nd collection date was 58.3 % (in comparison to the untreated control) and thus was higher than 40 %.

Earthworm abundance :

Table B.9.6.5-2 : Earthworm abundance in comparison to the untreated control

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	96.0	100.0	21.0	100.0	214.5	100.0	97.8	100.0
Agricultural control	68.8	71.6	12.8	60.7	109.5	51.0	64.0	65.5
Positive control	75.3	78.4	8.8*	41.7	177.5	82.8	68.3	69.8
300 L Metam-Sodium/ha	74.3	77.3	3.3*	15.5	74.8*	34.8	66.3*	67.8
1200 L Metam-Sodium/ha	81.5	84.9	2.8*	13.1	73.8*	34.4	54.5*	55.8

* statistically significantly different from the untreated control

AT : after treatment

Table B.9.6.5-3 : Earthworm abundance in comparison to the collection prior to application

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	96.0	100.0	21.0	21.9	214.5	223.4	97.8	101.8
Agricultural control	68.8	100.0	12.8	18.5	109.5	159.3	64.0	93.1
Positive control	75.3	100.0	8.8	11.6	177.5	235.9	68.3	90.7
300 L Metam-Sodium/ha	74.3	100.0	3.3	4.4	74.8	100.7	66.3	89.2
1200 L Metam-Sodium/ha	81.5	100.0	2.8	3.4	73.8	90.5	54.5	66.9

Table B.9.6.5-4 : Earthworm abundance in comparison to the agricultural control

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Agricultural control	68.8	100.0	12.8	100.0	109.5	100.0	64.0	100.0
300 L Metam-Sodium/ha	74.3	108.0	3.3*	25.5	74.8*	68.3	66.3	103.5
1200 L Metam-Sodium/ha	81.5	118.5	2.8*	21.6	73.8*	67.4	54.5	85.2

* statistically significantly different from the agricultural control

The earthworms collected in the **untreated control** within the experimental time are considered to represent the natural development of the earthworm population in this test site. The basic development observed in the untreated control of a decrease of earthworm numbers in the summer (June 2001), followed by an increase in autumn (October 2001) and slightly reduced numbers of earthworms in spring (May 2002), could be observed in all treatment groups.

The earthworm abundance in the **positive control** was statistically significantly reduced to 41.7 % of the untreated control in June 2001, thus fulfilling the guideline recommendations of an earthworm abundance reduction of 40–80 %.

Comparing the **agricultural control** with the untreated control shows that the mechanical soil cultivation has a great influence on the ability of an earthworm population to recover.

The total earthworm abundance in the treatment groups with **Metam-Sodium** showed a statistically significant reduction 3^{1/2} weeks after the application (June 2001) and 4^{1/2} months after application (October 2001), when compared to the untreated control and to the agricultural control.

One year after the application, the earthworm abundance in the **300 L Metam-Sodium/ha** treatment group was statistically significantly reduced compared to the untreated control (67.8 % of the untreated control). Since the values at experimental start were already different and the soil cultivation affected the earthworm population in addition, the comparison to the agricultural control is considered more appropriate showing a full recovery value of the 300 L Metam-Sodium/ha treatment group (103.5 % of the agricultural control). Considering the start value as a natural abundance in the plots treated with 300 L Metam-Sodium/ha, the values after one year also reach a good recovery level (89.2 % of the start value).

One year after the application, the earthworm abundance in the **1200 L Metam-Sodium/ha** treatment group was statistically significantly different compared to the untreated control (55.8 % of the untreated control) but was not statistically significantly different compared to the agricultural control (85.2 % of the agricultural control) and reached 66.9 % of the start value. The data show that the earthworm abundance in the 1200 L Metam-Sodium/ha group is slightly reduced one year after the application, but considering the whole development with constantly decreasing differences compared to the controls, the values of the last collection are regarded as an almost completed recovery process. It should be noted that the herbicidal impact of the test item (especially in the 1200 L Metam-Sodium/ha treatment group) caused an inhomogeneous plant cover before the 4th sampling which might also have influenced the process of recovery.

Earthworm biomass :

Table B.9.6.5-5 : Total earthworm biomass in comparison to the untreated control

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	g/m ²	(%)	g/m ²	(%)	g/m ²	(%)	g/m ²	(%)
Untreated control	41.1	100.0	9.3	100.0	62.9	100.0	39.9	100.0
Agricultural control	30.9	75.2	9.9	107.4	37.8	60.1	31.0	77.7
Positive control	31.4	76.4	2.5*	26.6	62.3	99.0	37.3	93.5
300 L Metam-Sodium/ha	38.1	92.6	4.6	49.8	39.2*	62.4	38.3	96.1
1200 L Metam-Sodium/ha	33.0	80.1	3.3	35.3	43.9*	69.8	26.6	66.7

* statistically significantly different from the untreated control

Table B.9.6.5-6 : Total earthworm biomass in comparison to the collection prior to application

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	g/m ²	(%)	g/m ²	(%)	g/m ²	(%)	g/m ²	(%)
Untreated control	41.1	100.0	9.3	22.5	62.9	152.9	39.9	96.9
Agricultural control	30.9	100.0	9.9	32.1	37.8	122.1	31.0	100.0
Positive control	31.4	100.0	2.5	7.8	62.3	198.1	37.3	118.6
300 L Metam-Sodium/ha	38.1	100.0	4.6	12.1	39.2	103.0	38.3	100.5
1200 L Metam-Sodium/ha	33.0	100.0	3.3	9.9	43.9	133.3	26.6	80.7

Table B.9.6.5-7 : Total earthworm biomass in comparison to the agricultural control

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	g/m ²	(%)	g/m ²	(%)	g/m ²	(%)	g/m ²	(%)
Agricultural control	30.9	100.0	9.9	100.0	37.8	100.0	31.0	100.0
300 L Metam-Sodium/ha	38.1	123.2	4.6	46.4	39.2	103.9	38.3	123.7
1200 L Metam-Sodium/ha	33.0	106.5	3.3	32.8	43.9	116.3	26.6	85.8

* statistically significantly different from the agricultural control

Earthworm biomass in the groups treated with Metam-Sodium showed a similar pattern of effect as for earthworm abundance. Biomass was less affected than earthworm abundance at the 2nd sampling (June 2001), where biomass decreased to 49.8 and 35.3 % of the untreated control (not statistically significant) or to 46.4 and 32.8 % of the agricultural control (not statistically significant).

4^{1/2} months after the application (October 2001), biomass values in the test item groups increased, minimizing the difference to the controls and representing 62.4 and 69.8 % of the untreated control (statistically significant) and 103.9 and 116.3 % of the agricultural control (not statistically significant). Based on the comparison with the agricultural control, the parameter of earthworm biomass reached the recovery level and the total biomass was also higher than the start values (103.0 and 133.3 % of the start values).

However, at the 4th sampling date (May 2002), the biomass value in the 300 L Metam-Sodium/ha treatment group fully recovered even if compared to the untreated control (96.1 % of the untreated control, not statistically significant). Also in comparison to the start value, recovery is confirmed (100.5 % of the start value). In the 1200 L Metam-Sodium/ha treatment group, the biomass value was slightly but not significantly reduced making 66.7 % of the untreated control but 85.8 % of the agricultural control and 80.7 % of the start value. As for abundance, this reduction could have been caused by the inhomogeneous plant cover.

Earthworm species composition :

The collected earthworms belonged to six epilobous and two tanylobous species. The most abundant species was *Aporrectodea caliginosa* with 20.0 ind/m² or 71.0 % of determined earthworms (mean over all treatment groups), followed by *Allolobophora chlorotica* 4.1 ind/m² or 14.4 % of determined earthworms and *Lumbricus castaneus* 3.5 ind/m² or 12.4 % of determined earthworms. 0.5 to 0.8 individuals of *Lumbricus terrestris* were found per m² at experimental start (2.0 % of determined species).

Table B.9.6.5-8 : Abundance of *Aporrectodea caliginosa* at each collection date

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	26.75	75.9	0.50	28.6	49.50	76.7	10.50	66.7
Agricultural control	17.25	70.4	1.00	44.4	30.25	81.8	8.75	76.1
Positive control	15.25*	63.5	0.00	0.0	46.75	83.1	13.25	77.9
300 L Metam-Sodium/ha	21.25	73.9	0.25	25.0	37.25	93.1	14.00	75.7
1200 L Metam-Sodium/ha	19.50	69.0	0.25	20.0	37.00	92.5	20.75	85.6

* statistically significantly different compared to the untreated control

Table B.9.6.5-9 : Abundance of *Allolobophora chlorotica* at each collection date

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	4.50	12.8	0.50	28.6	13.50	20.9	2.50	15.9
Agricultural control	3.00	12.2	0.25	11.1	4.75	12.8	2.00	17.4
Positive control	5.25	21.9	0.00	0.0	7.25	12.9	0.25	1.5
300 L Metam-Sodium/ha	0.75	2.6	0.00	0.0	1.25	3.1	2.50	13.5
1200 L Metam-Sodium/ha	6.75	23.9	0.00	0.0	0.25	0.6	2.50	10.3

* statistically significantly different compared to the untreated control

Table B.9.6.5-10 : Abundance of *Lumbricus castaneus* at each collection date

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	3.25	9.2	0.00	0.0	0.00	0.0	0.75	4.8
Agricultural control	3.50	14.3	0.00	0.0	0.00	0.0	0.00	0.0
Positive control	3.00	12.5	0.00	0.0	0.00	0.0	2.25	13.2
300 L Metam-Sodium/ha	6.00	20.9	0.00	0.0	0.00	0.0	0.00	0.0
1200 L Metam-Sodium/ha	1.75	6.2	0.00	0.0	0.00	0.0	0.50	2.1

* statistically significantly different compared to the untreated control

Table B.9.6.5-11 : Abundance of *Lumbricus terrestris* at each collection date

Treatment groups	Pre-sampling		3 ^{1/2} weeks AT		4 ^{1/2} months AT		1 year AT	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	0.75	2.1	0.50	28.6	1.00	1.6	1.25	7.9
Agricultural control	0.75	3.1	1.00	44.4	1.50	4.1	0.25	2.2
Positive control	0.50	2.1	0.75	50.0	1.25	2.2	1.00	5.9
300 L Metam-Sodium/ha	0.50	1.7	0.75	75.0	1.25	3.1	1.50	8.1
1200 L Metam-Sodium/ha	0.25	0.9	0.75	60.0	2.00	5.0	0.25	1.0

* statistically significantly different compared to the untreated control

The numbers of all species were reduced at the 2nd sampling (June 2001) in all treatment groups due to naturally occurring fluctuations. Based on the low abundances, comparison between the treatment groups is very difficult. For the abundance of *Aporrectodea caliginosa*, a high variation was observed in the Metam-Sodium groups in comparison to the controls, but this was regarded to be within the natural occurring range. *Lumbricus terrestris* was found in comparable densities in each treatment group at each collection date. *Lumbricus castaneus* completely disappeared at the 2nd and 3rd sampling date (June 2001 and October 2001) in all treatment groups and therefore no definite assessment for the 4th collection date (May 2002) can be made. However, this species was found in the untreated control and the 1200 L Metam-Sodium/ha treatment group, but not in the agricultural control, so the low numbers are considered not to be treatment related. For *Allolobophora chlorotica* abundance values in the Metam-Sodium treatment groups were reduced at the 2nd and 3rd sampling (compared to untreated or agricultural control) but recovered to values similar in the test item treated groups and the controls at the 4th collection (May 2002).

Earthworm juveniles :

Table B.9.6.5-12 : Abundance of juvenile earthworms at pre-sampling (May 2001)

Treatment groups	epilobous		tanylobous		total	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	37.5	39.1	23.3	24.2	60.8	63.3
Agricultural control	32.0	46.5	12.3	17.8	44.3	64.4
Positive control	38.0	50.5	13.3	17.6	51.3	68.1
300 L Metam-Sodium/ha	23.5	31.6	22.0	29.6	45.5*	61.3
1200 L Metam-Sodium/ha	37.8	46.3	15.5	19.0	53.3	65.3

Table B.9.6.5-13 : Abundance of juvenile earthworms at 3^{1/2} weeks after treatment (June 2001)

Treatment groups	epilobous		tanylobous		total	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	9.0	42.9	10.3	48.8	19.3	91.7
Agricultural control	7.3	56.9	3.3	25.5	10.5	82.4
Positive control	5.3	60.0	2.0	22.9	7.3	82.9
300 L Metam-Sodium/ha	1.8	53.8	0.5	15.4	2.3*	69.2
1200 L Metam-Sodium/ha	0.8	27.3	0.8	27.3	1.5*	54.5

Table B.9.6.5-14 : Abundance of juvenile earthworms at 4^{1/2} months after treatment (October 2001)

Treatment groups	epilobous		tanylobous		total	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	88.0	41.0	62.0	28.9	150.0	69.9
Agricultural control	38.5	35.2	34.0	31.1	72.5	66.2
Positive control	44.0	24.8	77.3	43.5	121.3	68.3
300 L Metam-Sodium/ha	10.5	14.0	24.3	32.4	34.8*	46.5
1200 L Metam-Sodium/ha	14.5	19.7	19.3	26.1	33.8*	45.8

Table B.9.6.5-15 : Abundance of juvenile earthworms at 1 year after treatment (May 2002)

Treatment groups	epilobous		tanylobous		total	
	ind/m ²	(%)	ind/m ²	(%)	ind/m ²	(%)
Untreated control	38.0	38.9	44.0	45.0	82.0	83.9
Agricultural control	13.5	21.1	39.0	60.9	52.5	82.0
Positive control	16.5	24.2	34.8	50.9	51.3	75.1
300 L Metam-Sodium/ha	11.8	17.7	36.0	54.3	47.8	72.1
1200 L Metam-Sodium/ha	9.8	17.9	20.5	37.6	30.3*	55.5

In the Metam-Sodium groups, the ratio of juveniles was reduced at the 2nd and 3rd sampling date (June 2001 and October 2001). The ratio of juveniles was not statistically significantly different at the 4th collection date (May 2002) in the 300 L Metam-Sodium/ha treatment group, but still showed a reduced ratio in the 1200 L Metam-Sodium treatment group, if compared to the untreated or agricultural control.

Conclusions of the RMS:

The study is acceptable.

The assessment of the effects of metam-sodium and its metabolite MITC should be done in comparison with the agricultural control only (same soil cultivation).

One year after treatment of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha), the earthworm abundance was 103.5 % and 85.2 % respectively of the agricultural control.

The earthworm biomass was 123.7 % and 85.8 % one year after application of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha) respectively.

Amongst the collected earthworms, the most abundant species was *Aporrectodea caliginosa*. 4^{1/2} months after treatment with 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha), the abundance of *Aporrectodea caliginosa* was comparable to that in the agricultural control.

The abundance of juvenile earthworms one year after application of 300 L Metam-Sodium/ha (equivalent to 152.1 kg a.s./ha) was comparable to the agricultural control, whereas at 1200 L Metam-Sodium/ha (equivalent to 608.4 kg a.s./ha) it was still statistically significantly lower.

In conclusion, one year after application of 152.1 kg a.s./ha, the earthworm population in the field has recovered. One year after application of 608.4 kg a.s./ha, there is a clear indication of an almost completed process of recovery. Since there was a reduced plant cover (lower food source) at 608.4 kg a.s./ha, this represents a worst case situation.

Added in August 2010:

Position paper to address the risk to earthworms from metam-sodium. (Brown K., 2009). Unpublished report

Earthworms abundance and species composition varies considerably between agricultural crops as differing amounts of organic matter are available for food. Crops grown in bare soil generally have fewer earthworms than those with ground cover. Earthworms in cultivated agricultural crops comprise both tanylobous and epilobous species. Tanylobous earthworms, such as *Lumbricus terrestris*, form U-shaped burrows and come to the surface to feed on leaf material and other organic matter which they take into their burrows. Epilobous species, such as *Aporrectodea caliginosa* live within horizontal tunnels which may be close to the soil surface. Due to the fact that their behaviour and feeding habits result in differing exposure to treatments the risk to epilobous and tanylobous worms are considered separately.

Toxicity :

The effects of metam-sodium on earthworms were evaluated in a field study (Luhrs, 2002) conducted in Germany in 2001 in accordance with the relevant guidelines (BBA VI (1994) and ISO 11268 (1997)). This study was evaluated in the DAR (2007) and in an addendum to the DAR (2008) and was considered to be valid and acceptable. Effects of metam-sodium applied at 300 L/ha⁻¹ and 1200 L/ha⁻¹ were compared with those of a toxic reference, an untreated control and a control which was cultivated in the same manner as the metam-sodium plots (i.e. which were rotavated after treatment to incorporate the test item). Earthworms were sampled (using an electrical extraction method) on one occasion before treatment and on three occasions up to 12 months after treatment. Adult worms were identified to species level and juveniles were recorded as being either epilobous or tanylobous.

The pre-treatment results confirmed that the study site supported adequate worm populations and that the most abundant species in samples from the site was the epilobous species *A. caliginosa*. Tanylobous adults were sampled in low numbers, as would be expected for bare soil plots, but epilobous and tanylobous juveniles were both well represented. Results for *A. caliginosa* adults and pre-adults show that neither metam-sodium treatment rate had any adverse effects on this species.

Effects of metam-sodium should be compared with those in the agricultural control, since this was also cultivated. When compared with the agricultural control there was a significant reduction in numbers of epilobous juveniles in both the 300 L/ha⁻¹ and 1200 L/ha⁻¹ metam-sodium-treatments after 3.5 months but no statistically significant differences ($P < 0.05$) were detected after 12 months.

Adults of tanylobous species were found in numbers which were too low to make a meaningful interpretation. Juvenile tanylobous worms were sampled in good numbers at the start of the study and were affected by metam-sodium (at both 300 and 1200 L/ha⁻¹) 3.5 weeks after treatment. Given the variability in the raw data, the fact that mean numbers across all treatments after twelve months exceeded those at the start of the study indicates that recovery had occurred. Statistical analysis of results for tanylobous juveniles after 12 months showed no significant differences ($P < 0.05$) between either the 300 L/ha⁻¹ or the 1200 L/ha⁻¹ metam-sodium treatments and the agricultural control.

Recovery :

The PRAPeR meeting (4 July 2008) considered the extent to which recovery observed in the field study was a function of the experimental plot size. Specifically, would recovery observed in 12.5 x 8 m plots represent recovery that would occur on a field scale.

The field study was conducted using plots with a surface area of 100 m², complying with the requirements of the accepted ISO and BBA guidelines. The acceptability of recovery within this test system will have been considered when approving the size of plots for use in this guideline.

Beyer (1992) reports that *A. caliginosa* burrows between 2.6 and 6.2 cm per day, giving an estimated annual burrowing distance of between 9.5 and 22.6 m assuming activity each day. It is therefore likely that plots 12.5 m x 8m would be fully recolonised by immigration from their margins within a 12 month period. Larger species, represented in this study by the tanylobous juveniles, can burrow between 12 to 27 cm per day (Martin, 1982) giving an annual recovery distance of 44 -98 m.

Tanylobous species would therefore be likely to recover on a field scale within one year. Although recovery by immigration for epilobous species would be slower, the most commonly found epilobous earthworm, *A. caliginosa*, was unaffected by metam-sodium. However, populations of earthworm species affected by metam-sodium would be expected to recover by reproduction of survivors as well as by immigration.

Conclusions :

Whilst metam-sodium at both 300 L/ha⁻¹ and 1200 L/ha⁻¹ had some impact on earthworm populations within a treatment season these effects were not of great magnitude and were transient. Populations of affected taxa had recovered by the following growing season. Recovery would be expected to occur on an agricultural scale by both immigration and reproduction.

Conclusion of the RMS :

From the field study (Lührs, 2002) it was concluded that one year after application of 152.1 kg a.s./ha, the earthworm population in the field has recovered.

One year after application of 608.4 kg a.s./ha, there is a clear indication of an almost completed process of recovery. Since there was a reduced plant cover (lower food sources) at 608.4 kg a.s./ha, this represents a worst case situation.

The notifier provided information on the migration distances of earthworm species, indicating that the recovery observed in the test plots is representative for the field situation.

In conclusion, the risk of metam-sodium and its metabolite MITC to earthworms is acceptable for the field use at the lower application rate of 153 kg a.s./ha and is sufficiently addressed for the higher application rate of 612 kg a.s./ha at the EU level (an almost completed process of recovery was demonstrated over a period of one year). RMS proposes that potential risk mitigation measures are taken into consideration at the Member State level for the higher application rates, allowing the earthworms to recover in the field.

B.9.6.6 Summary and risk assessment for earthworms (Annex IIIA 10.6.1)

First tier risk assessment for earthworms :

The risk assessment for earthworms is based on the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC of October 2002.

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil; the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is compressed with a roller. After a waiting period of 2 to 4 weeks, the soil is cultivated to allow the non-mineralised gasses to disperse. Since the product is phytotoxic, it is advisable to perform a germination test (Cress-test) on samples of soils collected from the treated field, before planting.

Earthworm populations are not exposed to the uses in greenhouse.

As a general soil fumigant, it is expected that metam-sodium will have initial effects on the entire soil fauna and flora, including earthworm populations. Therefore, the risk of metam-sodium and its metabolite MITC should be addressed in terms of recovery in the field.

An earthworm field study (Lührs U., 2002) was conducted from May 31, 2001 until May 22, 2002.

The assessment of the effects of metam-sodium and its metabolite MITC should be done in comparison with the agricultural control only (same soil cultivation).

One year after treatment of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha), the earthworm abundance was 103.5 % and 85.2 % respectively of the agricultural control.

The earthworm biomass was 123.7 % and 85.8 % one year after application of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha) respectively.

Amongst the collected earthworms, the most abundant species was *Aporrectodea caliginosa*. 4^{1/2} months after treatment with 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha), the abundance of *Aporrectodea caliginosa* was comparable to that in the agricultural control.

The abundance of juvenile earthworms one year after application of 300 L Metam-Sodium/ha (equivalent to 152.1 kg a.s./ha) was comparable to the agricultural control, whereas at 1200 L Metam-Sodium/ha (equivalent to 608.4 kg a.s./ha) it was still statistically significantly lower.

It was concluded that one year after application of 152.1 kg a.s./ha, the earthworm population in the field has recovered.

One year after application of 608.4 kg a.s./ha, there is a clear indication of an almost completed process of recovery. Since there was a reduced plant cover (lower food source) at 608.4 kg a.s./ha, this represents a worst case situation.

The notifier provided information on the migration distances of earthworm species, indicating that the recovery observed in the test plots is representative for the field situation.

In conclusion, the risk of metam-sodium and its metabolite MITC to earthworms is acceptable for the field use at the lower application rate of 153 kg a.s./ha and is sufficiently addressed for the higher application rate of 612 kg a.s./ha at the EU level (an almost completed process of recovery was demonstrated over a period of one year). RMS proposes that potential risk mitigation measures are taken into consideration at the Member State level for the higher application rates, allowing the earthworms to recover in the field.

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

Collembola test :

Extended laboratory study on the effects of Metam sodium on reproduction of the Collembola *Folsomia candida* exposed to soil residues aged under field conditions. (Meister A., 2002).

Guidelines :

ISO 11267 ISO Soil Quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants, 1999

GLP :

Yes

Material and Methods :

Test substance : Metam-Sodium, formulation containing 507 g a.s./L, batch n° : G30013149

Test species : *Folsomia candida* (Collembola : Isotomidae)

Number of organisms, life stage, age : 5 replicates each with 10 springtails, juveniles and adults, 10 – 12 days old

Type of test : extended laboratory test

Test substrate :

Control and test item : natural soil from an arable field close to Rossdorf (Germany), silty sand to extremely loamy sand, 5.7 – 13.7 % clay, 31.1- 39.0 % silt, 51.4 – 57.4 % sand, 0.69 – 1.20 % organic carbon, < 0.1 % CaCO₃, soil pH : 5.2, maximum water capacity : 42.97 %

Positive control : artificial soil : 69.5 % fine quartz sand, 20 % kaolinite clay, 10 % sphagnum-peat, 0.5 % CaCO₃

Applied concentrations :

control (untreated natural soil); positive control (200 mg Betosip (166 g/L phenmedipham)/kg artificial soil (dry weight)) only used for the bioassay; test item (1 x 1200 L Metam-Sodium/ha (equivalent to 608.4 kg a.s./ha))

The application of the test item was done on May 31, 2001. The application was done directly onto the soil surface according to agricultural practice. Immediately after application of each plot the test item was incorporated with a rotavator (dept of this rotavation treatment : approximately 15 – 25 cm) and the soil was compressed with a roller. The soil surface of the test item treated plots was re-opened on June 13, 2001. The test plots were mulched on June 30, 2001. *Phacelia* sp. was sown on July 07, 2001 to cover the soil surface.

The soil was aged under field conditions for 55 days. Soil samples were taken with a soil sampler of approximately 5 cm diameter up to 10 cm depth on July 25, 2001. From each of the 4 plots per treatment, 10 soil samples were taken from different locations (40 samples per treatment group) and were mixed afterwards. The soil was sieved to 5 mm. To minimize the influence of predators occurring in the soil, the samples were defaunated. Therefore, the field soil was frozen for 2 days, kept at room temperature for 10 days and was frozen again for 3 days. The water amount of the soil was adjusted to 40-60 % of its maximum water holding capacity with deionised water.

Exposure route :

The exposure units were glass vessels, closed tightly by plastic-lids to avoid water evaporation, filled with approximately 30 g ± 5 % (wet weight) natural soil (control and test item) or artificial soil (positive control). The adult test organisms were exposed to the test item for 28 days. The number of living adult Collembola at 28 days after application was counted. The number of juvenile Collembola at 28 days after application was counted under binocular microscopes.

Feeding : After introduction of the test organisms, approximately 2 mg of granulated dry yeast was spread over the soil surface at the beginning of the test. Granulated dry yeast was added *ad libitum* weekly. The food was moistened by a drop of water.

Test conditions :

temperature : 19 – 21 °C

pH : 5.2 (at start), 5.0 – 5.7 (at end)

water content : 14.8 – 20.2 % (at start), 39.7 – 55.2 % (at end) of the maximum water holding capacity

photoperiod : 16/8 hours light/dark cycle

light intensity : 470 – 756 lux

Findings :Table B.9.7-1 : Summary of effects of the formulation Metam-Sodium 507 g a.s./L on *Folsomia candida* in an extended laboratory test

Evaluation criteria	Control	Positive control	608.4 kg a.s./ha
Mortality of adult Collembola after 4 weeks	0.0 % $\pm$ 0.0 %	42.0 % $\pm$ 8.4 %*	14.0 % $\pm$ 8.9 %*
Number of Collembola after 4 weeks	244 $\pm$ 87	323 $\pm$ 64	810 $\pm$ 126*
Reproduction relative to control (%)	-	+ 32.4	+ 232

* statistically significantly different from the control

No abnormal behaviour or conditions were observed with the surviving Collembola.

All reproduction data were statistically analysed with a test for identifying outliers (Dixon, 1953). In the field soil treated with 1200 L Metam-Sodium/ha, an outlier was identified (replicate 2: 1617 juvenile Collembola). This value was therefore not considered.

The coefficient of variation for reproduction was not met (35.65 % instead of 30 % at maximum). As this study was performed with field soils and as field soils are much more heterogeneous than the artificial soil used normally for Collembola studies, this value was still acceptable.

The reproduction in the control was very low. In the study, the mean reproduction in the control was 244 $\pm$ 87 juvenile Collembola. Normally, the reproduction in the artificial control soil ranges between 400 to 1000 juvenile Collembola. For this test, the soil was taken from the non-cultivated control plot of the earthworm field study. The soil surface was very dry and hard when the samples were taken. To improve the conditions for the Collembola during the study, the soil was sieved to 5 mm.

The test item plots treated with 1200 L Metam-Sodium/ha showed an increased reproduction of Collembola compared to the control (810 $\pm$ 126 juvenile Collembola). The test item treated plots were cultivated several times (rotavating on May 31, 2001, June 13, 2001; mulching on June 30, 2001; sowing of *Phacelia* on July 07, 2001). These procedures loosened the field soil. Therefore, the increased reproduction in the treated plots might be a consequence of the cultivation which probably enabled a much more suitable soil structure and a better growth of fungi which are the main food source of *Folsomia candida*.

The reproduction in the positive control (323 $\pm$ 64 juvenile Collembola) is comparable to other tests performed in artificial soil. Since the reproduction in the control was very low, the reproduction in the positive control showed an increase compared to the control. But the sensitivity of the test system was already demonstrated by the mortality occurring in the positive control.

Conclusions of the RMS:

The study is not acceptable.

The reproduction of Collembola in the control was very low due to inappropriate soil conditions. The assessment of the effects of metam-sodium and its metabolite MITC should be done in comparison with an agricultural control (same soil cultivation) and not with an untreated control. Therefore, the results of this extended laboratory study are inconclusive.

First tier risk assessment for non-target soil macro-organisms :

The risk assessment for non-target soil macro-organisms is based on the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC of October 2002.

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil; the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is compressed with a roller. After a waiting period of 2 to 4 weeks, the soil is cultivated to allow the non-mineralised gasses to disperse. Since the product is phytotoxic, it is advisable to perform a germination test (Cress-test) on samples of soils collected from the treated field, before planting.

As a general soil fumigant, it is expected that metam-sodium will have initial effects on the entire soil fauna and flora, including Collembola populations. Therefore, the risk of metam-sodium and its metabolite MITC should be addressed in terms of recovery in the field. An extended laboratory study is only able to assess the potential for recolonisation, but not the actual recovery. Therefore, a semi-field or field study should demonstrate that the field can be recolonised with Collembola from the border stripes (off-field) or underlying soil layers (in-field). This new field study can be integrated with the requested semi-field or field study for the non-target arthropods.

Non-target soil macro-organisms are not exposed to the uses in greenhouse.

Added in August 2010:

A field trial to determine the effects of METAM-SODIUM on the non-target arthropod fauna of arable land in SW-France after one application in spring. (Aldershof S., 2010).
Additional argumentation for recovery of Collembola and Gamasida.

See study summary under B.9.5.3.

Collembola:

The most abundant suborder, Arthropleona, collected from soil core samples, were initially reduced after injection with METAM-SODIUM. They remained adversely affected during the entire season, but from autumn onwards densities were similar to the control, indicating full recovery (Figure B.9.7-1). The suborder Symphypleona collected from soil core samples were much less abundant (Figure B.9.7-2). Initially no treatment related adverse effect was observed. During and after soil injection numbers were low both in the test item treatment and in the control treatment. Only later in the season numbers collected in METAM-SODIUM treated plots were lower than numbers found in control plots, but differences were never statistically significant.

Collembola collected with pitfalls were not identified beyond order level. They showed a transient but statistically significant reduction up to two months after application (Figure B.9.7-3). Treatment effects on collembolans sampled from the soil layer were less persistent than effects deduced from populations living within the soil. Full recovery was observed in June 2008, two months after treatment. Next season samples confirmed these findings.

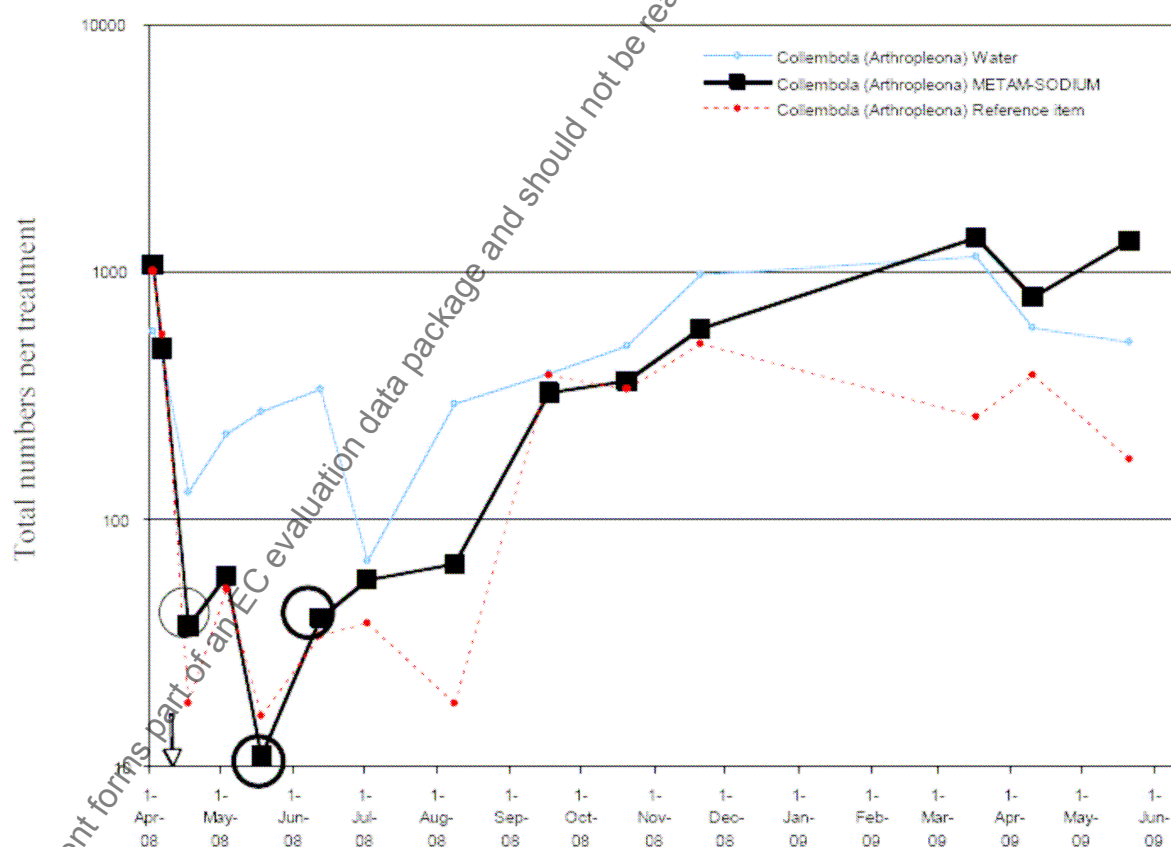


Figure B.9.7-1 : Density graphs Arthropleona (Collembola) (soil samples)

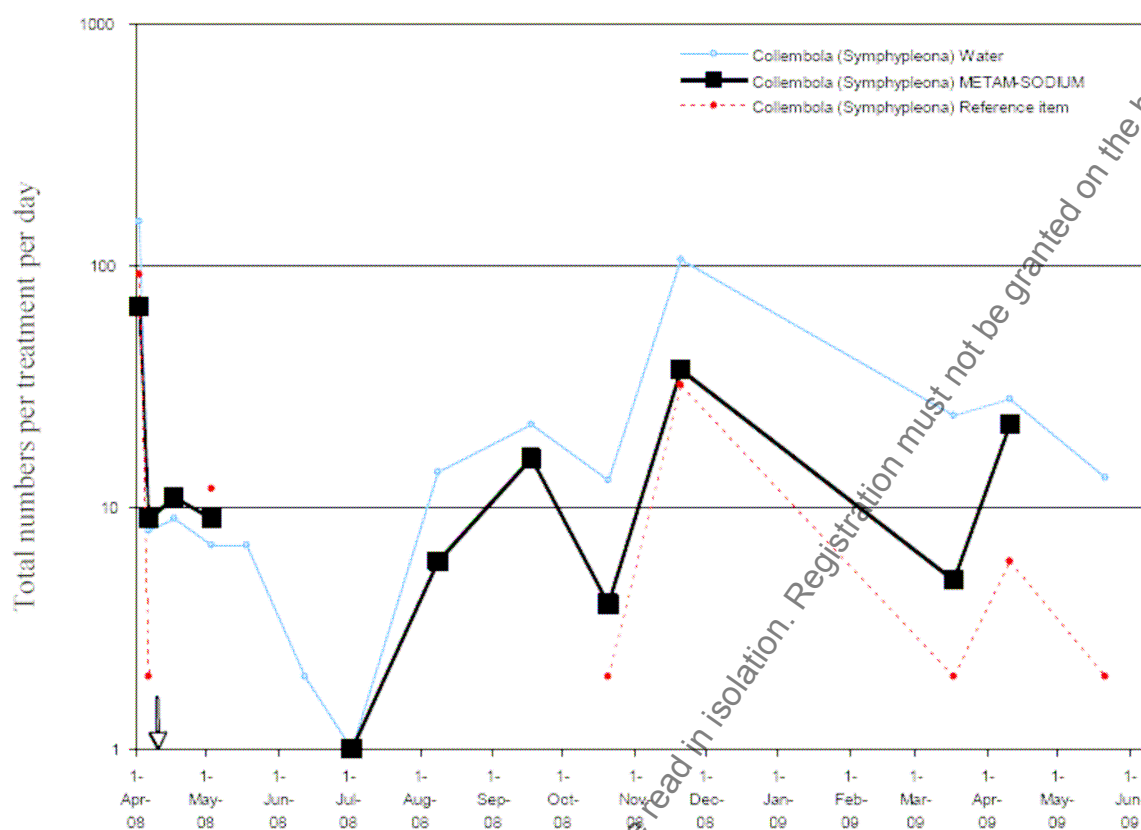


Figure B.9.7-2 : Density graphs Symphypleona (soil samples)

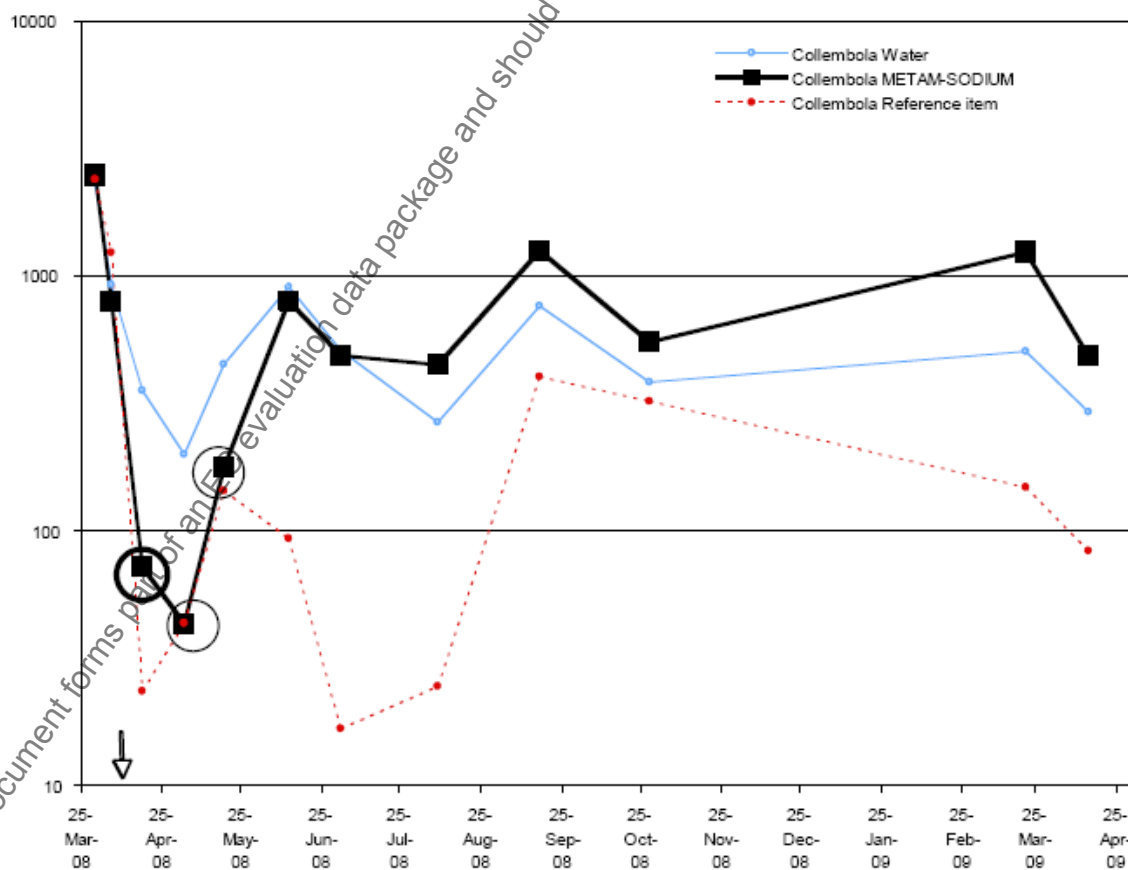


Figure B.9.7-3 : Density graphs total Collembola (pitfall samples)

Gamasida :

Gamasida were initially adversely and statistically significantly affected by the test item treatment immediately following injection (Figure B.9.7-4: all stages pooled, Figure B.9.7-5: female stages and Figure B.9.7-6: juveniles and males). Recovery was observed to take place within two months after injection, but densities of both adult and juvenile stages were significantly reduced again in August and September 2008. Populations had recovered at the end of the season (November 2009).

In spring 2009 female densities were statistically significantly lower than the control on one sampling moment (10 April). However, this did not result in considerable reductions of juvenile populations (Figure B.9.7-5), hence it is unlikely that Gamasida populations would be reduced later in the season. On the last sampling moment in May 2009 differences compared to the control in adult and juvenile populations were not statistically significant and had reduced to ecologically acceptable levels below 50%, implying recovery of Gamasida populations in METAM-SODIUM treated plots.

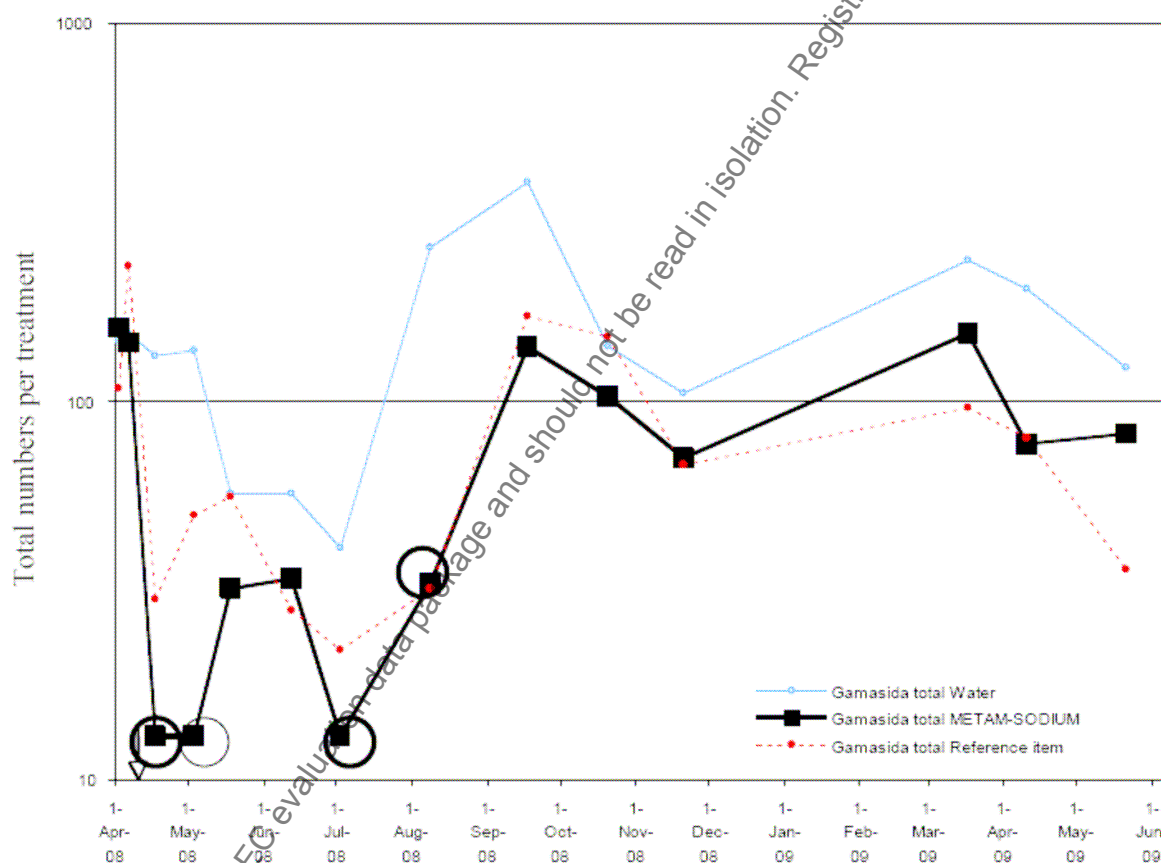


Figure B.9.7-4 : Density graphs total Gamasida (Acari) (soil samples)

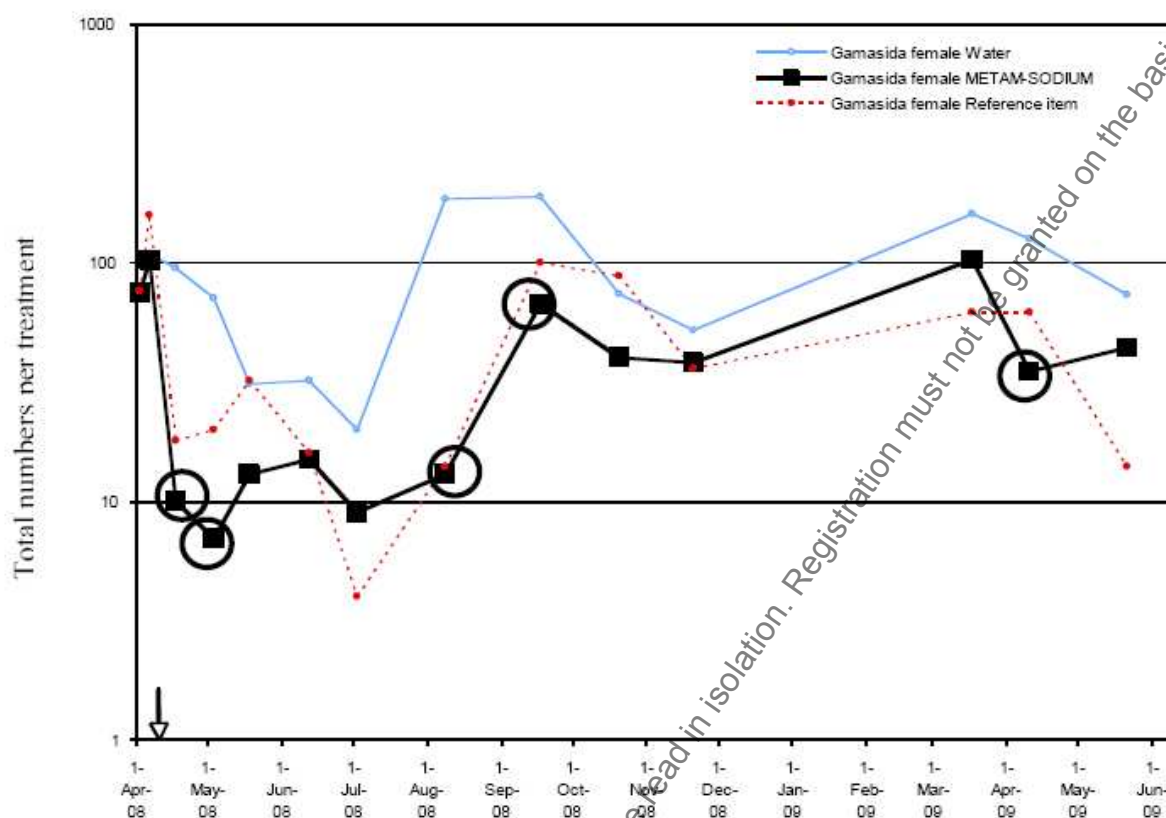


Figure B.9.7-5 : Density graphs Gamasida females (Gamasida, Acari) (soil samples)

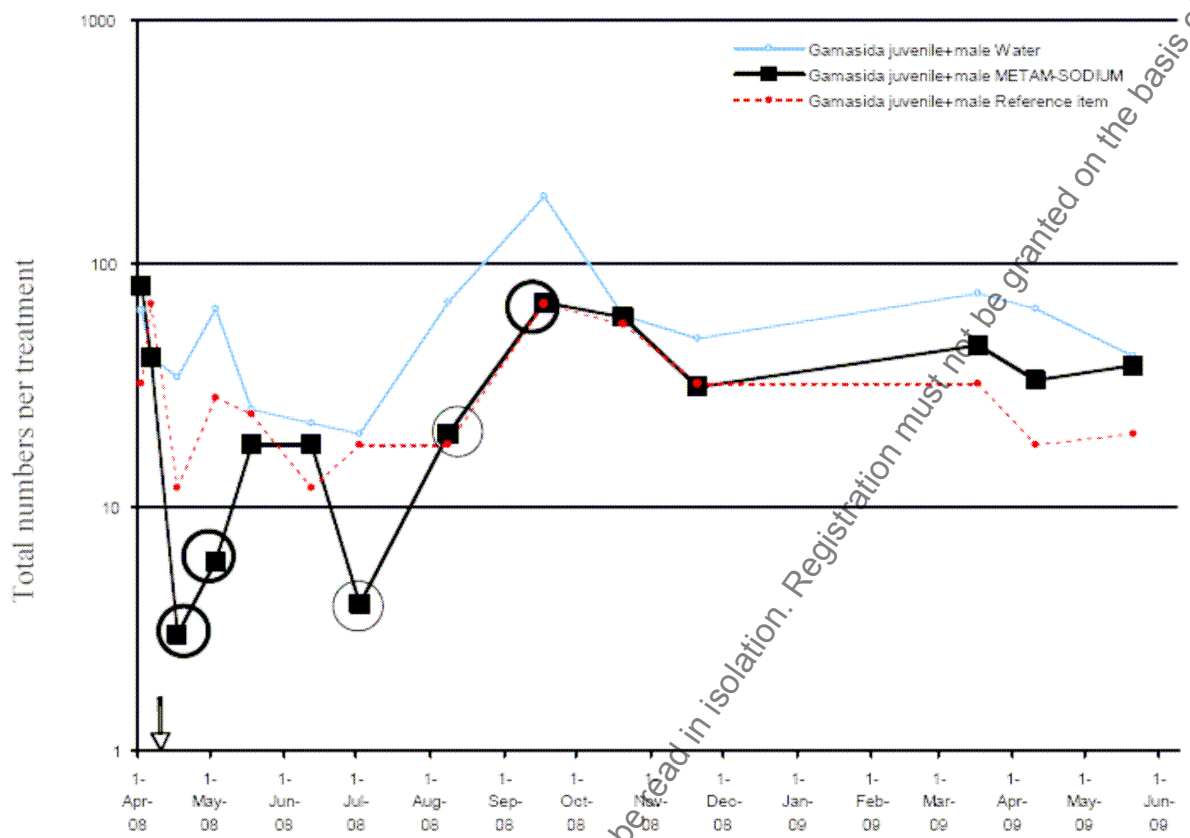


Figure B.9.7-6 : Density graphs Gamasida juveniles and males (Gamasida, Acari) (soil samples)

Conclusions of the RMS :

There is no evidence from any of the density graphs in the field study of Aldershof (2010) that long-term effects on soil dwelling macro-invertebrates occurred due to metam-sodium treatment. All affected soil dwelling invertebrates had fully recovered in abundance within the same season and no adverse effects extended into the year after treatment.

The risk of metam-sodium and its metabolite MITC to non-target soil macro-organisms is acceptable for the intended field uses since recovery within 1 year after application was demonstrated (see also B.9.5.4).

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

B.9.8.1 Impact of the active substance on soil microbial activity (Annex IIA 8.5)

Not required. A laboratory study with the formulation Metam-Sodium 507 g a.s./L was performed. Metam-sodium is used as a soil fumigant, therefore a significant impact on the soil microbiological activity is inherent to the product. Standard laboratory tests on nitrogen transformation and carbon mineralisation are thus deemed to be not relevant. An extended laboratory study/field study was conducted instead, examining the recovery of the soil after treatment.

B.9.8.2 Impact of the formulations on soil microbial activity (laboratory) (Annex IIIA 10.7.1)

Field study to evaluate the effects of Metam sodium on the activity on the soil microflora. (Reis K.H., 2002).

Guidelines :

EPPO Decision-making scheme for the environmental risk assessment of plant protection chemicals. Chapter 7: Soil microflora. EPPO Bulletin 24: 1-16, 1994

SETAC Guideline : Procedures for assessing the environmental fate and ecotoxicity of pesticides, soil micro-organisms, p. 40-42, March 1995

OECD Guideline 216 : Soil Microorganisms : Nitrogen Transformation Test (2000)

OECD Guideline 217 : Soil Microorganisms : Carbon Transformation Test (200)

GLP :

Yes

Material and Methods :

Test substance : Metam-Sodium, formulation containing 507 g a.s./L, batch n° : G130013149

Test species : soil micro-organisms as contained in the biologically active field soil

Type of test : laboratory test with soil samples collected from the field

Earthworm field test :

Test site : The study was carried out on an arable field test site, situated in Darmstadt-Dieburg, Rossdorf in Germany. The size of the test area was ca 5000 m². In 1998 winter wheat and in 1999 winter barley was cultivated. In 2000, the field was uncultivated after harvest of winter barley.

Test design : The experiment was of randomized block design with 4 treatments (untreated control, agricultural control, treatment with 300 L Metam-sodium/ha and treatment with 1200 L Metam-sodium/ha), each with 4 replicates. Each plot measured 100 m² (12.5 m x 8 m) with 3 m distance between the plots in the rows and 7 m distance between the plots in the columns. One plot of each treatment group was fixed for use in the soil microflora study.

Treatments :

- untreated control
- agricultural control (to check the mechanical impact)
- single treatment at 300 L Metam-Sodium/ha (152.1 kg a.s./ha)
- single treatment at 1200 L Metam-Sodium/ha (608.4 kg a.s./ha)

One week before application (May 24, 2001), a few weeds were cut and left in the field. The application of Metam-Sodium was on bare soil on May 31, 2001. After application, each plot was immediately treated with a rotavator to incorporate the test item up to a depth of 25 cm and compressed with a roller. Two weeks after the application (June 13, 2001), the soil was re-opened with a cultivator. Another soil cultivation was performed on July 7, 2001 using a rotary harrow to a depth of 5-8 cm. During this last cultivation, 10 kg/ha *Phacelia tanacetifolia* was sown using a seed drill.

In the agricultural control the same soil cultivation was performed. The positive control and the untreated control were not cultivated.

During the growing season, the plant cover in the Metam-Sodium treated groups and the agricultural control was more or less equal. In winter 2001/2002, *Phacelia tanacetifolia* was killed by frost (in every plot) and the soil was only covered by the remains of the dead *Phacelia tanacetifolia* and the wild weeds. Since the density of wild weeds in the test item groups (and especially in the 1200 L/ha group) was lower than in the agricultural control,

the test site did not ensure exactly the same conditions for every treatment group until the fourth sampling though in spring 2002 fallen off *Phacelia tanacetifolia* seeds were growing again. This means that in winter 2001/2002 between the third and the fourth sampling the plant cover in the test item group (especially in the 1200 L/ha group) was lower than in the controls. The reduced plant cover provides a lower source of food and is therefore representing worst case conditions.

Soil microflora laboratory test :

Soil type : silty/loamy sand

- untreated control : silty sand, 6.5 % clay, 39.0 % silt, 54.5 % sand, 0.69 % organic carbon, < 0.1 % chalk, 41.8 – 51.7 % of maximum water holding capacity
- 300 L Metam-Sodium/ha : silty sand, 7.7 % clay, 37.1 % silt, 55.2 % sand, 0.99 % organic carbon, < 0.1 % chalk, 41.8 – 51.7 % of maximum water holding capacity
- 1200 L Metam-Sodium/ha : loamy sand, 9.5 % clay, 33.1 % silt, 57.4 % sand, 0.91 % organic carbon, < 0.1 % chalk, 41.8 – 51.7 % of maximum water holding capacity

Soil sampling collection dates from the field :

Soil samples were taken from the untreated control and the Metam-Sodium treated plots on following days :

pre-evaluation (- 6 days) : May 25, 2001

1 day after application : June 01, 2001

28 days after application : June 28, 2001

56 days after application : July 26, 2001

102 days after application : September 10, 2001

184 days after application : November 29, 2001

Additionally, at day 148 after application (October 24, 2001), a sampling of the control and the agricultural control plots was made. At day 184 after application (November 29, 2001), all control plots and the Metam-Sodium treated plots were sampled by mix samples (5 individual samples per plot were mixed to one sample)

Sampling scheme : The samplings were done by means of a trowel taking soil from hole (depth approximately 5 cm to 15 cm) after the sod was removed. An excessive amount of soil was taken and carefully sieved to 5 mm grain size.

Sampling method : 5 individual soil samples (replicates) were taken from 1 plot of each treatment group (control, 300 L Metam-Sodium/ha and 1200 L Metam-Sodium/ha). They were taken from different locations of the plot : 4 samples were taken from each edge of the square (approximately 1 m) and one sample of the centre.

Positive control : Only in the laboratory a positive control was made. At day 0 (field application of Metam-Sodium), a soil sample was carefully sieved at a grain size of 5 mm. The soil was moistened to approximately 50 % of the maximum water holding capacity. The final concentration was 75 mg Dinoterb/kg soil dry weight.

Test conditions :

temperature : 20 – 22 °C

pH : 7.3 (- 6 days in control) – 6.2 (until day 184), 7.3 – 6.1 (Metam-Sodium treated plots)

water content : control : 11.4 % (start), 16.9 % (end), 6.2 % (min), 16.9 % (max)

300 L/ha : 12.7 % (start), 17.8 % (end), 8 % (min), 17.8 % (max)

1200 L/ha : 11.8 % (start), 18.7 % (end), 5.3 % (min), 18.7 % (max)

light regime : in the dark

Exposure time : 184 days (for untreated control and Metam-Sodium treated plots)

102 days (for the positive control)

Nitrogen mineralisation and nitrification : Determination of the concentration of NH_4^+ , NO_2^- and NO_3^- was done following extraction from the soils with 0.1 M KCl-solution. The determination of ammonium was by means of an ion sensitive electrode and controller. The determination of nitrite and nitrate was by means of liquid chromatography.

Soil amendment : 5 g glucose/kg moist soil for glucose induced respiration rate

Soil respiration rate : The measurement of the soil respiration was carried out by means of a pressure sensor. The sensor records the pressure in an isolated test system. Amounts of consumed oxygen are equivalent to the formed CO_2 . The CO_2 is trapped (by KOH). Therefore the pressure decrease is directly linked to the respiration rate. The carbon dioxide release (oxygen consumption) was measured up to 24 consecutive hours.

Findings :

Table B.9.8.2-1 : Influence of the formulation Metam-Sodium 507 g a.s./L on nitrogen transformation (individual samples)

Incubation (days)	Total NO ₃ ⁻ - N (mg N/100 g dry weight) and % deviation from the untreated control				
	Control	300 L Metam-Sodium/ha		1200 L Metam-Sodium/ha	
	(mg N/100 g)	(mg N/100 g)	(%)	(mg N/100 g)	(%)
- 6	0.788	1.308*	+ 66.0	0.584	- 25.9
1	1.178	1.760	+ 49.4	0.682	- 42.1
28	0.520	3.098*	+ 496	1.508*	+ 190
56	0.628	4.826*	+ 688	3.012*	+ 380
102	0.290	0.076	- 73.8	0.038	- 86.9
184	0.112	0.156	+ 39.3	0.226	+ 102

* statistically significantly different from the untreated control

Incubation (days)	Total N _{min} (mg N/100 g dry weight) and % deviation from the untreated control				
	Control	300 L Metam-Sodium/ha		1200 L Metam-Sodium/ha	
	(mg N/100 g)	(mg N/100 g)	(%)	(mg N/100 g)	(%)
- 6	1.222	2.390	+ 95.6	0.888	- 27.3
1	1.700	2.538*	+ 49.3	1.662	- 2.24
28	0.842	4.646*	+ 452	3.378*	+ 301
56	0.912	5.676*	+ 522	3.898*	+ 327
102	0.550	0.336	- 38.9	0.298	- 45.8
184	0.372	0.416	+ 11.8	0.486	+ 30.6

* statistically significantly different from the untreated control

N_{min} : sum of NH₄⁺-N, NO₂⁻-N and NO₃⁻-N

To determine the differences between the untreated control and the agricultural control (mechanical impact) plots, two of each were tested at 148 days after application. Differences in nitrate content of up to 60 % are observed between the different controls. Also, individual differences between replicates within one plot are frequently above 50 %, which leads to the conclusion that the trigger value used in the laboratory studies which accepts only 25 % difference between treated and control soil, is not applicable to field conditions.

Table B.9.8.2-2 : Influence of the formulation Metam-Sodium 507 g a.s./L on nitrogen transformation (mix samples) at day 184

Evaluation criteria	Control	Agricultural control	300 L/ha	1200 L/ha
NO ₃ ⁻ -N (mg N/100 g soil d.w.)	0.14 ± 0.01	0.23 ± 0.06	0.24 ± 0.04	0.28 ± 0.04
N _{min} (mg N/100 g soil d.w.)	0.14 ± 0.01	0.23 ± 0.06	0.24 ± 0.04	0.36 ± 0.04
Deviation of plot samples	34.1	45.2	46.2	45.9
% of untreated control	0	+ 64.3	+ 71.4	+ 100.0
Significance to untreated control	-	*	*	*
% of agricultural control	- 39.1	0	+ 4.35	+ 21.7
Significance to agricultural control	n.s.	-	n.s.	n.s.

N_{min} : sum of NH₄⁺-N, NO₂⁻-N and NO₃⁻-NDeviation of plot samples calculated as : ((Value_{max} - Value_{min}) x 100) / Value_{max}

* statistically significantly different

n.s. not statistically significantly different

There was a statistically significant difference between the Metam-Sodium treated plots and the untreated control plots but not with the agricultural control plots. This indicates that the mechanical impact of agricultural machines cultivating the soil had a tolerable effect on the nitrogen turnover in the agricultural plots. The impact on the nitrogen content is therefore mainly caused by the mechanical impact of the agricultural machines and not by Metam-Sodium.

Table B.9.8.2-3 : Influence of the formulation Metam-Sodium 507 g a.s./L on carbon transformation (individual samples)

Incubation (days)	Mean microbial respiration rate (mg CO ₂ /100 g soil d.w./h) and % deviation from the untreated control				
	Control	300 L Metam-Sodium/ha		1200 L Metam-Sodium/ha	
	(mg/100 g/h)	(mg/100 g/h)	(%)	(mg/100 g/h)	(%)
- 6	1.958	1.782	- 8.99	2.018	+ 3.06
1	1.600	0.560	- 65.0	0.460	- 71.3
28	2.073	2.007	- 3.18	2.425	+ 17.0
56	1.394	0.873	- 37.4	1.170	- 16.1
102	1.290	0.621	- 51.9	1.000	- 22.5
184	0.534	0.359	- 32.8	0.711	+ 33.1

To determine the differences between the untreated control and the agricultural control (mechanical impact) plots, two of each were tested at 148 days after application. Differences in respiration rate between controls of up to 36 % are observed. Also individual differences between replicates ranged from 17 % to 36 % (mostly above 30 %). This leads to the conclusion that the trigger value used in the laboratory studies, which accepts only 25 % difference between treated and control soil, is not applicable to field conditions.

Table B.9.8.2-4 : Influence of the formulation Metam-Sodium 507 g a.s./L on carbon transformation (mix samples) at day 184

Evaluation criteria	Control	Agricultural control	300 L/ha	1200 L/ha
mg CO ₂ /100 g soil d.w./ha	0.643 ± 0.15	0.549 ± 0.10	0.446 ± 0.09	0.598 ± 0.11
Deviation of plot samples	37.0	35.3	35.5	34.2
% of untreated control	0	- 14.6	- 30.6	- 7.00
Significance to untreated control	-	n.s.	n.s.	n.s.
% of agricultural control	+ 17.1	0	- 18.8	+ 8.93
Significance to agricultural control	n.s.	-	n.s.	n.s.

Deviation of plot samples calculated as : $((\text{Value}_{\max} - \text{Value}_{\min}) \times 100) / \text{Value}_{\max}$

* statistically significantly different

n.s. not statistically significantly different

The study was valid since the positive control Dinoterb treated soil differed by more than 25 % from the control in the carbon mineralisation test and the soil nitrogen turnover test. The differences were statistically significant different.

Conclusions of the RMS :

The study is acceptable although high variations were observed between replicates from the untreated control and the agricultural control.

A soil microflora laboratory test was conducted with soil samples from the earthworm field study with application rates of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha). The effects of metam-sodium and its metabolite MITC on nitrogen and carbon transformation were transient and the processes were not drastically impaired.

The notifier submitted also two publications :

Effect of soil fumigation with Vapam on the dynamics of soil microflora and their related biochemical activity. (Sinha A.P., Agnihotri V.P., Kishan Singh, 1979).

Some literature is available describing the effect of metam (MITC) on non-target soil microflora. Sinha *et al.* (1979) investigated the effects of metam-sodium on the dynamics of soil microflora.

Fungal propagules, in general, were drastically reduced in metam-sodium amended soils. The toxicant from 250 to 1000 ppm was lethal to *Fusarium* spp. Only in the early part of the experiment, higher concentrations of metam-sodium appreciably reduced the population of bacteria and actinomycetes, though later on, their population gradually increased. The numbers of *Azotobacter* in soil amended with 125, 250 and 500 ppm did not alter appreciably, but their population in treated soils increased over the check on the 45th day. All concentrations of metam-sodium lowered the population of *Rhizobia*.

Metam-sodium produced inhibitory effect on soil nitrification, inhibition period varying with the amount of chemical applied initially. metam-sodium from 125 to 500 ppm stimulated the ammonification process, while its higher concentration (1000 ppm) produced detrimental effect for 15 days. The toxicant increased CO₂ production for first 32 days.

Results suggest that extraordinary success of metam-sodium in controlling soil-borne infections of *Pythium* and *Rhizoctonia* was not entirely due to its fungicidal action. Partly it was also due to an increase in the population of known antagonists of these pathogens in fumigated soils.

Evolution of the fungus flora following different soil treatments. (Welvaert W., 1974).

Welvaert (1973) studied the evolution of soil flora after different types of soil treatment. One of the treatments was fumigation in greenhouses with 100 L/ha metam-sodium.

Soil samples were analyzed for the presence of soil fungi before, immediately after treatment and 1, 3 and 12 months after treatment. For maximum one month, growth of the fungi was inhibited. Afterward the population recovered. It was found that after chemical disinfection, repopulation occurs generally more quickly than after steaming. Especially the antagonists of parasitic organisms recovered well, such as *Penicillium* spp., which gives rise to synergistic action called "induced antagonism".

Conclusions of the RMS:

These publications do not allow evaluating the effects of metam-sodium and its metabolite MITC on non-target soil micro-organisms.

B.9.8.3 Further laboratory, glasshouse or field-testing to investigate impact on soil microbial activity (Annex IIIA 10.7.2)

Not required.

B.9.8.4 Summary of studies on soil non-target micro-organisms – exposure and risk assessment for soil non-target micro-organisms

Risk assessment for non-target soil micro-organisms :

The risk assessment for non-target soil micro-organisms is based on the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC of October 2002.

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation **under a plastic film** on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation **under a plastic film** on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation **under a plastic film** on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation **under a plastic film** on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil; the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is compressed with a roller. After a waiting period of 2 to 4 weeks, the soil is cultivated to allow the non-mineralised gasses to disperse. Since the product is phytotoxic, it is advisable to perform a germination test (Cress-test) on samples of soils collected from the treated field, before planting.

As a general soil fumigant, it is expected that metam-sodium will have initial effects on the entire soil fauna and flora, including non-target soil micro-organisms. Therefore, the risk of metam-sodium and its metabolite MITC should be addressed in terms of recovery in the field.

A soil microflora laboratory test (Reis K.H., 2002) was conducted with soil samples from the earthworm field study with application rates of 300 and 1200 L Metam-Sodium/ha (equivalent to 152.1 and 608.4 kg a.s./ha). The effects of metam-sodium and its metabolite MITC on nitrogen and carbon transformation were transient and the processes were not drastically impaired.

Non-target soil micro-organisms are not exposed to the uses in greenhouse.

In conclusion, the risk of metam-sodium and its metabolite MITC to non-target soil micro-organisms is acceptable for the intended uses.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6; Annex IIIA 10.8)

Not required.

First tier risk assessment for non-target terrestrial plants :

The risk assessment for non-target terrestrial plants is based on the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC of October 2002.

The intended uses of metam-sodium are :

- carrot : 1 x 408 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- corn salad : 1 x 306 kg a.s./ha, field, soil injection + incorporation at 15 cm by rotavation
- cucumber : 1 x 459 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- eggplant/aubergine : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- pepper : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- potato : 1 x 153 kg a.s./ha, field, soil injection + incorporation at 25 cm by rotavation
- strawberry : 1 x 612 kg a.s./ha, field, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, greenhouse, drip-irrigation under a plastic film on the planting row
- tomato : 1 x 612 kg a.s./ha, field, soil injection at 5 cm + coverage by 10 cm of soil
- grape : 1 x 1020 kg a.s./ha, field, soil injection at 40 cm at the site of the de-rooted vine (0.2 L/m²)

The application of metam-sodium is on bare soil; the product is incorporated into the soil (soil injection or drip irrigation) and thereafter the soil is compressed with a roller. The mode of application excludes the off-field exposure.

In conclusion, the risk of metam-sodium and its metabolite MITC to non-target terrestrial plants is acceptable for the intended uses.

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

Activated sludge respiration inhibition test of Metam-sodium. (Van der Kerken K., 2002).

Guidelines :

OECD Guideline 209 : Activated Sludge : Respiration Inhibition Test

EPA Guideline : 712-C-96-168 : OPPTS 850.6800 – modified activated sludge, respiration inhibition test for sparingly soluble chemicals

Directive 87/302/EEC : Part 11.C : Methods for Determination of Ecotoxicity : Biotic Degradation – Activated Sludge : Respiration Inhibition Test. Official Journal of the European Communities L133 of 30.05.1988

“A statistical procedure for modelling continuous toxicity data”, Bruce and Versteeg. Environmental Toxicology and Chemistry, vol. 11, pp. 1485-1494, 1992

GLP :

Yes

Material and Methods :

Test substance : Metam-Sodium, formulation containing 512 g a.s./L, batch n° 32 E 28/6

Test species : activated sludge from domestic sewage treatment

Type of test : The inhibitory effect of metam-sodium on the oxygen consumption of activated sludge suspension was determined for a contact time of 3 hours.

Microbial inoculum : 4 g/L mixed liquor suspended solids level, pH : 7.39

Test design : 2 replicates for the water control, 1 replicate per treatment

Applied concentrations :

control; positive control (3,5-dichlorophenol); 0.1, 0.32, 1.0, 3.18, 10.1, 32.0 mg a.s./L

Test conditions :

temperature : 18.4 – 19.3 °C

pH : 7.59 – 7.77 (start), 7.66 – 7.99 (end)

Findings :

Inhibitory effects : The inhibition after 3 hours contact time was 1.8 %, 9.1 %, 30 %, 51 %, 64 % and 69 % at the treatment levels of 0.1, 0.32, 1.0, 3.18, 10.1 and 32.0 mg a.s./L.

Validity : Respiration rates for the two control vessels were comparable.

Reference substance : The calculated EC₅₀ of 3,5-dichlorophenol was 6.30 mg/L after 3 hours.

Conclusions :

The study is acceptable.

Endpoints :

EC₅₀ (activated sludge, 3 hours) = 4.36 mg a.s./L

B.9.11 Batches used for the ecotox studies

Added in June 2008 and accepted by PRAPeR 53:

The applicant provided an overview of all ecotoxicological studies and available information on the test items used. This is presented in Volume 4 of the DAR under C.1.2.3 on p. 24-26.

RMS considers that information submitted is sufficiently addressing the issue on ecotox batches.

Added in August 2010:

Ecotoxicological data on DMTU

After the meeting of experts (July 2008) EFSA identified a data gap to provide ecotoxicological data to address the equivalence of the relevant impurity DMTU compared with metam-sodium.

Statement of the notifier:

N,N'-dimethylthiourea is a minor impurity in the end product. An overview of the ecotoxicological studies and available information of the test item used is given in the Volume 4 of the DAR. For all sources of metam-sodium and metam-potassium test substance, it was demonstrated or it can reasonably be expected that DMTU was present as an impurity in the study. The ecotoxicological properties of DMTU are therefore covered by studies on metam-sodium / metam-potassium.

Basic ecotoxicological properties of DMTU are available and can be compared with the properties of metam. For chronic endpoints, MITC is a better basis for comparison as metam is very rapidly degraded into MITC. The comparison below clearly shows that the impurity DMTU is less toxic than the active substance metam-sodium.

Table B.9.11-1 : Comparison of the ecotoxicity between the active substance metam, its metabolite MITC and the impurity DMTU

	Metam	MITC	DMTU*
Bioaccumulation	Not bioaccumulable		Not bioaccumulable
Fish : <i>Brachydanio rerio</i>			LC ₅₀ , 7 days: 465 mg/L
Fish: <i>Oncorhynchus mykiss</i>		NOEC 28 d: 0.004 mg/mL	LC ₅₀ , 60 days: 135 mg/L
<i>Daphnia magna</i>	EC ₅₀ 48 h: 0.99 mg a.s./L		EC ₅₀ , 48 hours: 193 mg/L
<i>Daphnia</i> (immobilisation)		NOEC reproduction: 0.00625 mg/L	EC ₅₀ , 21 days: 59 mg/L

* Ref: Material Safety Data Sheet; Ekaland DMTU CR-CB (2007)

Conclusion of RMS:

Information on the ecotoxicological batches is presented in Volume 4 of the DAR.

It can reasonably be expected that DMTU was also present as an impurity in the test items used in the ecotoxicological studies and therefore the ecotoxicological properties of DMTU are expected to be covered by the studies on metam-sodium or metam-potassium.

Furthermore, a comparison of some acute and chronic ecotoxicological endpoints of DMTU with those of metam and MITC, respectively, indicates that DMTU is clearly less ecotoxic than the active substance.

Finally, in the environmental fate section it was demonstrated that DMTU is not persistent, the geomean DT_{50} of DMTU in soils being 0.23 (0.15-0.35) days. This implies that no long-term exposure to DMTU is to be expected. Taking the above considerations into account, the exposure to DMTU is limited, the ecotoxicity of DMTU is assessed by the available data package and the risk assessment of DMTU is covered by the risk assessment for metam and MITC. Therefore, RMS concludes that the risk of DMTU to non-target organisms is acceptable.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

B.9.12 References relied on**B.9.12.1 Ecotoxicology of the active substance (Annex IIA 8)**

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 8.1.1 /01	Munk R	1985-12	Avian single dose oral LD50 Metam sodium (aqueous solution) to the Bobwhite quail BASF AG, 86/0521 Not GLP Not published	Y	USA TF
IIA 8.1.2 /01	Pedersen CA, Solatycki AM	1993-09-01	Metam Concentrate: 8 Day acute Dietary LC50 study in Bobwhite quail Bio-life, 127-001-01 GLP Not published	Y	USA TF
IIA 8.1.2 /02	Pedersen CA, Solatycki AM	1993-09-01	Metam Concentrate: 10 Day Acute Dietary LC50 Study in Mallard Ducklings Bio-Life, 127-002-02 GLP Not published	Y	USA TF
IIA 8.2.1 /03	Howard DJ, Johnston CD	1971-01-25	Vapam: Safety evaluation on Rainbow trout Woodard Research Corporation, RIC0658 Not GLP Not published	Y	USA TF
IIA 8.2.1 /04	Zok S	2002-06-25	Methylisocyanate (metabolite of BAS 002 N, dazomet) acute toxicity study on the rainbow trout (<i>Oncorhynchus mykiss</i>) in a semistatic system over 96 hours BASF AG, 2002/1006168 GLP Not published	Y	KST, Taminco
IIA 8.2.1 /05	Schupner J, Stachura B	1991-09-12	W150 MITC: The acute toxicity of MITC Technical to Rainbow trout, <i>Oncorhynchus mykiss</i> , in a flow through system Nor-Am Chemical Company, 502AF GLP Not published	Y	Bayer CropScience
IIA 8.2.1 /06	Gelbke HP, Munk R	1986-04	Report on the study of Acute Toxicity of Metam sodium in the Bluegill (<i>Lepomis Macrochirus</i> Raf.) BASF AG Ludwigshafen, 86/0512 Not GLP Not published	Y	USA TF
IIA 8.2.1 /08	Howard DJ, Johnston CD	1970-12-30	Vapam safety evaluation on Bluegill sunfish Woodard Research Corporation T-2173 Not GLP Not published	Y	USA TF

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 8.2.1 /09	Schupner J, Stachura B	1991-09-04	W149 MITC: The acute toxicity of MITC Technical to Bluegill sunfish, <i>Lepomis macrochirus</i> , in a flow through system Nor-Am Chemical Company, 501AF GLP Not published	N	Bayer CropScience
IIA 8.2.1 /11	Howard DJ, Johnston CD	1970-12-14	Vapam Safety evaluation on <i>Cyprinodon variegatus</i> (Broad Killifish) Woodard Research Corporation, T-2175 Not GLP Not published	Y	USA TF
IIA 8.2.1 /12	Howard DJ, Johnston CD	1970-12-07	Vapam Safety Evaluation on Striped <i>Morone saxatilis</i> Woodard Research Corporation, T-2174 Not GLP Not published	Y	USA TF
IIA 8.2.2.1/01	Munk R	1990-02-23	Sublethal toxic effects on rainbow trout (<i>Salmo gairdneri</i> RICH. = <i>Oncorhynchus mykiss</i>) of Methylisothiocyanat (MITC) BASF AG, 42F0761/895215, BASF 90/10055 GLP Not published	Y	KST, Taminco
IIA 8.2.4 /01	Bias, Merz	1985-05	Determination of the Acute toxicity of BASF 005 00 N metam sodium 510 g/l to the Waterflea <i>Daphnia Magna</i> Straus BASF AG, 85/0497 Not GLP Not published	Y	USA TF
IIA 8.2.4 /03	Dohmen GP	2002-07-22	Effect of Methylisothiocyanate on the immobility of <i>Daphnia magna</i> STRAUS in a 48-hour semi-static, acute toxicity test BASF AG, 58330, BASF 2002/ 1006188 GLP Not published	Y	KST, Taminco
IIA 8.2.5 /01	Jatzek	2001-11-30	Methylisothiocyanat – Determination of the chronic effect on the reproduction of the water flea <i>Daphnia magna</i> STRAUS BASF AG, 99/0547/51/2BASF 2002/ 1000250 GLP Not published	Y	KST, Taminco
IIA 8.2.6 /01	Jenkins CA	2003-05-14	Metam sodium 510 g/l. Algal growth inhibition assay Huntingdon, UCB 833/023032 GLP Not published	Y	Taminco, Buckman
IIA 8.2.6 /02	Kubitza J	1998-09-15	Effect of Methyl isothiocyanate on the Growth of the Green Alga <i>Pseudokirchneriella subcapitata</i> BASF AG 48881, BASF 98/10767 GLP Not published	Y	KST, Taminco

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA 8.2.6 /03	Kubitza J	2002-07-03	Effect of Methylisothiocyanate on the growth of the blue-green alga <i>Anabaena flos-aquae</i> BASF AG, 106691 –BASF 2002/ 1006170 GLP Not published	Y	KST, Taminco
IIA 8.2.8 /01	Junker M	2002-07-18	Effect of methyl isocyanate (metabolite of BAS 002 N, Dazomet) on the growth of <i>Lemna gibba</i> BASF AG, 121437, 2002/ 1006181 GLP Not published	Y	Taminco, KST
IIA 8.3.2 /01	Drexler A	2002-03-20	Extended laboratory study on the effects of Metam-Sodium on reproduction of rove beetles <i>Aleochara bilineata</i> exposed to soil residues aged under field conditions Ibacon, 9204071 GLP Not published	Y	Taminco, FMC Foret, Lainco
IIA 8.4.2 /01	Lühns U	2002-09-19	Field Study to Evaluate the Effects of Metam-sodium on Earthworms Ibacon, 9201023 GLP Not published	Y	Taminco, FMC Foret, Lainco
IIA 8.4.2/02	Lühns U.	2007-07-19	Statement to Final Report: Field Study to Evaluate the Effects of Metam-sodium on Earthworms Ibacon, 9201023 GLP Not published	Y	Taminco
IIA 8.5 /01	Reis K-H	2002-05-31	Field study to Evaluate the Effects of Metam-Sodium on the Activity of the Soil Microflora Ibacon, 9202080 GLP Not published	Y	Taminco
IIA 8.7 /01	Van der Kerken K	2002-05	Activated sludge respiration inhibition test of Metam Sodium Lisec, WE-09-044 GLP Not published	Y	Taminco, Buckman, FMC Foret, Lainco

B.9.12.2 Ecotoxicology of the formulations (Annex IIIA 10)

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIIA 10.1.2/01	Noordam A, Bakker F	2010-02-01	Bird censuses on sterilized and unsterilized carrot plots in Les Landes, France during spring 2008. MITOX Consultants Unpublished Study number R-T006BIR GLP: no Not published	Y	Taminco
IIIA 10.1.4/01 IIIA 10.3/02	Bakker F; Roig J	2010-02-05	Metam sodium: A field study to determine residues in carrot field invertebrates as potential food items for birds and small mammals in SW France. Mitox Consultants Report N° T004IRW GLP:yes Not published	Y	Taminco
IIIA 10.1/01. IIIA 10.3/03	Brown KC	2010-01-26	The acute Risk to Birds and Mammals potentially exposed following proposed commercial use of Metam Sodium No report number GLP : no Not published	Y	Taminco
IIIA 10.2 /01	Farrelly E.	2004-11-22	Aquatic Risk Assessment for Metam-sodium and MITC Exponent International Limited Not published Not GLP	Y	Taminco
IIIA 10.3/01.	Bakker F, Reich T	2010-01	Small mammal survey on sterilized and unsterilized carrot plots and at the field perimeter in a commercial arable field in Les Landes, France during spring 2008. MITOX Unpublished study number R-T005SMA. GLP: no Not published	Y	Taminco
IIIA 10.5.2/01 IIIA 10.6.2/01	Aldershof S.	2010-01-13	A field trial to determine the effects of METAM-SODIUM on the non-target arthropod fauna of arable land in SW-France after one application in spring MITOX Consultants, Amsterdam, The Netherlands Report No. T003FFW GLP: yes Not published	Y	Taminco
IIIA 10.6.1/01	Brown K.	2009	Position paper to address the risk to earthworms from metam-sodium Ecotoxicologist and Environmental Consultant, Plymouth, Devon, PL7 4EB England GLP: no Not published	Y	Taminco

Annex point / reference number	Author(s)	Date	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
10.6.2/02	Bakker F	2010	Bakker F.(2010) A field trial to determine the effects of METAM-SODIUM on the non-target arthropod fauna of arable land in SW-France after one application in spring. Additional argumentation for recovery of Collembola and Gamasida. MITOX Consultants Unpublished Study Number T003FFW (additional argumentation) GLP: yes Not published	Y	Taminco
10.9/01	Anonymous	2007	Material Safety Data Sheet Ekaland DMTU CR-CB Version : 1 Not GLP Published	N	Public