ENTOMELA 50SL/ENT50

DOCUMENT M-CP, Section 10

ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 10 ECOTOXICOLOGICAL STUDIES ON PLANT PROTECTION PRODUCTS

Introduction

PHYTOPHYL manufactures “Hydrolysed Protein” which is made of Beet molasses and Urea. Both of them are used very widely for many years and have not ever classified as dangerous substanceBeet molasses are a natural by-product of the sugar industry, defined as the end product of sugar manufacture or refining from which no more sugar may be economically crystallized by conventional means.

Beet molasses mainly used for two purposes, Animal feed additive and Alcohol Production.

There is no evidence in bibliography that Beet molasses are for some reason toxic, irritant or ecologically unsafe.

PHYTOPHYL & FORESTRY COMISSION notified urea according to 91/414 and the substance is now approved under Reg. (EC) No 1107/2009. No toxicity studies were submitted but literature data about the toxicity of urea indicated limited toxicological potential.

During this first notification and inclusion Urea was not registered to ECHA but now has a full registration, the dossier is evaluated and there are 163 active registrants as a high volume chemical (production of 10.000 000 – 100.000.000 TONNES per year).

The annual application rate for urea, or hydrolysed protein in case of ENTOMELA 50SL for 6 applications per year according to the table of intended uses (CP 3.3) is:

Application rate per year for each active substance and total nitrogen content (6 applications/year)	
Hydrolysed protein	1.8kg – 2.08 kg/ha
Urea	0.576 kg – 0.648kg kg/ha
Total nitrogen content	0,288-0.333kg/ha

These rates are very low if we compare them to the annual application rates for urea as fertilizer which are reported to the ECHA site and are 60kg, 120kg, 180kg N/ha.

We can see that the use of Nitrogen fertilizers emits 180-540 times more nitrogen to the environment than the use of ENTOMELA 50SL for bait sprays and the quantities of urea and beet molasses that liberated to the environment are very low in comparison to the use of similar compounds as fertilizer or other uses, or even the quantities of them in wastewater of human origin.

An additional provision of the approval of hydrolysed proteins (amending Implementing Regulation EU No 540/2011) was that the applicant submits to the Commission confirmatory information as regards: The risk to aquatic organisms.

PHYTOPHYL with the other two applicants for the inclusion of Hydrolysed protein prepared a literature review in a private Spanish Laboratory IRTA. On concluding remarks of this study refers that “There is therefore no evidence for any adverse effects of hydrolysed proteins on aquatic ecosystems in general and on aquatic organisms in particular”.

PHYTOPHYL submit a DRR for ENTOMELA 50SL on 2015 according to reg. 1107/2009 and below are the Overall comments of zRMS on Ecotoxicological studies section:

zRMS overall comments:

Hydrolysed proteins (beet molasses hydrolysate)

No toxicity studies on non-target organisms were submitted during the EU peer review of the active substance hydrolysed proteins. However, according to the Draft Assessment Report for hydrolysed proteins (Volume 3, Annex B-9: Ecotoxicology, April 2008) due to the nature of the active substance and the characteristics regarding its fate and behaviour in the environment (biodegradable, non-persistent, non bioaccumulative), the use of hydrolysed proteins is considered of low danger for the terrestrial and aquatic wildlife and ecosystem in general. In fact, the available data indicate that it may be expected that hydrolysed proteins do not have any unacceptable influence on the environment.

However, two points regarding the environmental effects and risk assessment were raised during the EU peer review of the active substance hydrolysed proteins (EFSA Journal 2012; 10(2):2545): i) The necessity of toxicity studies on aquatic organisms directly related to the classification and labelling and ii) The requirement of environmental exposure assessments to soil, surface water, aquatic sediment and groundwater. Depending on the outcome of these assessments (whether the exposure to the environment arising from the representative uses is greater than the natural background level) risk assessments to non-target organisms (birds and mammals, aquatic organisms, bees, non-target arthropods, non-target soil organisms, non-target plants) may be required.

In order to address the former point, the three Notifiers for the inclusion of hydrolysed proteins in Annex I to Council Directive 91/414/EEC (BIOIBERICA S.A., PHYTOPHYL – N.G. STAVRAKIS, SICIT 2000 S.p.A.) have provided confirmatory data which are presented in the Addendum IV to DAR (Volume 3, Annex B-9: Ecotoxicology, September 2014). The EU evaluation of these confirmatory data is currently under progress. Based on the submitted data the designated RMS (Greece) has reached the following conclusion:

Although no specific testing toxicity data on either of the hydrolysed proteins notified (animal tissue hydrolysate, beet molasses urea hydrolysate, collagen protein hydrolysate) have been submitted, taking into account:

- *(i)* the lack of any information *or evidence in the scientific literature related to the aquatic toxicity potential of hydrolysed proteins,*
- *(ii) the indication of low hazard and risk associated with the use of hydrolysed proteins (e.g. beet molasses-urea hydrolysate) in insect attractants for bait spray applications compared to other nitrogen compounds (e.g. fertilizers) and*
- *(iii) the nature of the active substance and its characteristics regarding the fate and behaviour in the environment (biodegradable, non-persistent, non bioaccumulative),*

it can be concluded that the use of hydrolysed proteins is of low danger for the aquatic ecosystems in general and for the aquatic organisms in particular. In consequence, from the RMS’s point of view, hydrolysed proteins should not be assigned any classification for aquatic hazards and should be deemed *as non-dangerous for the environment substances.*

It should be also noted that the applicant (PHYTOPHYL – N.G. STAVRAKIS) has provided a “Genetically Modified Organisms Free” certificate to the zRMS which ascertains and confirms that the starting material for the preparation of hydrolyzed proteins (beet molasses) is not obtained from/does not contain genetically modified plants.

Urea

A considerable amount of information on the ecotoxicological potential of urea based on reviews by other organizations (US EPA, SIDS, IUCLID database) has been made available during the EU peer review of the active substance urea. The available information indicate limited toxicological potential and low ecotoxicity to non-target organisms. In addition, studies on the environmental fate and behaviour of urea clearly demonstrate that urea is highly soluble and biodegradable (both in soil and aquatic compartment), and it is not subject to accumulation within the ecosystem (log P_{OW} = -1.59 at 20-25°C). It has to be also noted that urea has been applied to growing arable crops and to pasture as one of the commonest nitrogen fertilisers for many decades, with few contra-indications. It is thus perhaps not surprising that assessments of the toxicity of the substance would not suggest it is hazardous by nature (DAR, Volume 3, Annex B-9: Ecotoxicology, April 2008).

Regarding aquatic environment, the available information was considered sufficient taking into account the nature of the active substance and that all of the toxicity values provided were consistent of low toxicity to aquatic organisms. In addition, biodegradation is expected to be the major fate process of urea in the aquatic ecosystem. Overall the proposed use of urea was considered to pose low risk to aquatic organisms (Addendum I to DAR, Annex B-9: Ecotoxicology, June 2011).

However, two points regarding the environmental effects and risk assessment were raised during the EU peer review of the active substance urea (EFSA Journal 2012;10(1):2523): i) The necessity of submission of the original ecotoxicological studies presented in the DAR and more specifically of the aquatic toxicity studies directly related to the classification and labelling of the active substance (the information available was limited to summaries of US EPA and OECD assessments and the IUCLID database), ii) The requirement of risk assessments to non-target organisms pending on the finalization of the environmental exposure assessments of urea and its transformation products (consideration of whether the exposure to the environment arising from the representative uses of the active substance is greater than the natural background level). This requirement is relevant for spray uses to conifer stumps and olive trees.

Taking into account the available information on the two active substances, the intended uses of ENT50 (soluble concentrate of urea with beet molasses) in spot bait spray applications is not expected to pose any unacceptable effects to non-target organisms (birds, aquatic organisms, wild mammals, bees, non-target arthropods, earthworms and other soil-macroorganisms, soil microorganisms and non-target plants) and no testing toxicity data are required.

All above mentioned justify that according to recent scientific knowledge there is no need for further eco-toxicological studies about the risk of Beet molasses-Urea hydrolysate and particularly for the p.p.p. ENTOMELA 50SL.

In next paragraphs we are giving also ECHA presented data about eco-toxicological risk bout the active substance urea.

CP 10.1 Effects on Birds and Other Terrestrial Vertebrates

“Urea is of inherently low toxicity and is rapidly assimilated into the nitrogen cycle by soil microorganisms; exposure is therefore limited.

The effects of long term use of urea fertiliser at 60, 120 and 180 kg N/ha/year was assessed on lumbricid earthworms in uncultivated turfgrass on loamy sand soil. The test sites were treated twice yearly for 20 years. Urea fertiliser reduced earthworm numbers and biomass and lowered pH. It was concluded that application of nitrogenous fertilisers for long periods may have a deleterious effect on earthworms in the absence of liming (Wei-Chum et al, 1990).

Low phytotoxicity is predicted for urea: the substance is widely used as a plant nutrient (N-source) in fertiliser, hence toxicity is unlikely.

The results of a study in soy bean plants confirm the low toxicity of urea.

Urea is of inherently low toxicity to microorganisms as it is utilised as a nutrient and nitrogen source. Testing of toxicity to soil microorganisms is scientifically unjustified.

The limited data available indicate that urea is of low toxicity to birds. A waiver is proposed for this endpoint on grounds of exposure.”

CP 10.1.1 Effects on birds

Risk assessment for birds

CP 10.1.1.1 Acute oral toxicity

CP 10.1.1.2 Higher tier data on birds

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Risk assessment for other terrestrial vertebrates

CP 10.1.2.1 Acute oral toxicity to mammals

CP 10.1.2.2 Higher tier data on mammals

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

CP 10.2 Effects on Aquatic Organisms

Risk assessment for aquatic organisms

Below is the summary and the remarks from IRTA study submitted on 2013 as confirmatory data for hydrolysed prpoteins:

Author: Institute for Food and Agricultural Research and Technology (IRTA)

Title: Bibliographical analysis of the effects of hydrolysed proteins on aquatic organisms

Executive summary

The objective and scope of this study was to make a complete and systematic technical and scientific bibliographical review of the effects that the use of protein hydrolysate baits may have on the aquatic organisms.
We present here the report of such a study, detailing the databases, search engines and open access repositories and catalogues consulted, as well as the search strategy performed (i.e. key words used and the Boolean operators applied to combine them).
The outcome of this review is that there is no evidence of any adverse effects of protein hydrolysate baits on aquatic organisms.

Concluding remarks

After a systematic bibliographical search as detailed above, no effects (of any kind) of the use of hydrolysed protein baits on aquatic organisms were found in the database and open access information resources consulted. No bibliographical records in relation to this subject were found. There is therefore no evidence for any adverse effects of hydrolysed proteins on aquatic ecosystems in general and on aquatic organisms in particular. *A priori*, hydrolysed proteins should be of low toxicological concern, since by definition they consist of the amino acid building blocks found in all living organisms and no risk to aquatic organisms is expected from their use as insect attractant inplant protection products. Furthermore, it should be noted that the quantities of hydrolysed proteins (such as animal tissue, urea, collagen protein and beet molasses hydrolysates) that are used as spray bait to attract insects are very low in comparison to the amounts used in other applications (e.g in fertilizers).

No extra data are presenting only for a.s. urea below is the **ECHA endpoint summary** on Aquatic toxicity:

Urea is of very low acute toxicity to aquatic organisms.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

No extra data for the p.p.p. are presented only for a.s. urea below is the ECHA endpoint summary on Aquatic toxicity:

Toxicity to fish

The 48 hour LC50of urea in golden orfe is reported to be >10000 mg/l. This can also be considered as the NOEC. The results reported by the two laboratories were identical. The effects of urea on survival, food utilization and oxygen consumption of the fresh water fish Oreochromis mossambicus were studied. The percentage survival of O. mossambicus when exposed to different concentrations of urea at 24, 48, 72 and 96 h exposures was noted and it was found that 22000 and 38000 mg/L urea concentration were sublethal and lethal, respectively. The median lethal concentration, which killed 50% of the fish during 96 h exposure, was 28000 mg L^{-1}. Rearing the fish in increasing sublethal concentrations of urea, it was found that the feeding rate decreased from 34.341 ± 7.067 mg g live $fish^{-1}$ d^{-1}(control) to 13.921 ± 2.315 mg g live $fish^{-1}$ d^{-1}at the highest concentration of urea (22,000 mg L^{-1}). Growth rate was drastically reduced. The consumption of oxygen in O. mossambicus diminished from 0.962 ± 0.208 to 0.645 ± 0.118 mg g live $fish^{-1}$ h^{-1} when reared in the highest sublethal concentration of urea. The 96 hour LC50of urea to B. barnawas > 9100 mg/l. The NOEL was 4961 ppm. The 96 hour acute LC50of urea to golden orfe fish is reported in a further study to be >6810 mg/l. No long-term toxicity data are available: a waiver is proposed for this endpoint. Urea is of inherently low toxicity to fish species: it is a normal product of protein catabolism and therefore fish have evolved effective excretion mechanisms. Additionally, exposure will be limited by the action of microorganisms and incorporation of urea into the nitrogen cycle.

Toxicity to aquatic invertebrates

The 24 hour EC50 for urea in Daphnia was reported to be >10000 mg/l; urea is not acutely toxic to daphnids. The 24 hour LC50 values for freshwater snail eggs, juveniles and adults were reported to be 14241 mg/l, 18255 mg/l and 22998 mg/l. Following 48 hours exposure, the LC50value for adults was calculated to be 13477 mg/l. In another study, the 24 hour LC50values for eggs, juvenile and adult snails were reported to be 13532 mg/l, 24504 mg/l and 26024 mg/l, respectively. Following 48 hours exposure, the LC50value for adults was calculated to be 21412 mg/l. It is concluded that, under normal laboratory conditions, urea displays low molluscicidal activity. The 4 hour LC50 in mosquito (Aedes aegypti) larvae is reported to be 60000 mg/l. No long-term toxicity data are available: a waiver is proposed on exposure grounds. Urea is of inherently low toxicity to species of aquatic invertebrates and exposure will be limited by the action of microorganisms and incorporation of urea into the nitrogen cycle

Toxicity to algae

The 192 hour toxicity threshold of blue-green algae urea was 47 mg/l. To some extent urea exhibits toxic action to Microcystis aeruginosa. The 7 day toxicity threshold of urea to Scenedesmus quadricauda was >10000 mg/l. The 72 hour toxicity threshold of Entosiphon sulcatum to urea was 29 mg/l, and the 16 hour toxicity threshold of urea to Pseudomonas putida was > 10000 mg/l.

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

CP 10.2.3 Further testing on aquatic organisms

CP 10.3 Effects on Arthropods

CP 10.3.1 Effects on bees

Risk assessment for bees

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

CP 10.3.1.1.2 Acute contact toxicity to bees

CP 10.3.1.2 Chronic toxicity to bees

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

CP 10.3.1.4 Sub-lethal effects

CP 10.3.1.5 Cage and tunnel tests

CP 10.3.1.6 Field tests with honeybees

CP 10.3.2 Effects on non-target arthropods other than bees

Risk assessment for other non-target arthropods

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

CP 10.3.2.3 Semi-field studies with non-target arthropods

CP 10.3.2.4 Field studies with non-target arthropods

CP 10.3.3 Other routes of exposure for non-target arthropods

CP 10.4 Effects on Non-Target Soil Meso- and Macrofauna

CP 10.4.1 Earthworms

Risk assessment for earthworms

CP 10.4.1.1 Earthworms – sub-lethal effects

CP 10.4.1.2 Earthworms – field studies

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

CP 10.4.2.1 Species level testing

CP 10.4.2.2 Higher tier testing

CP 10.5 Effects on Soil Nitrogen Transformation

Risk assessment for Soil Nitrogen Transformation

CP 10.6 Effects on Terrestrial Non-Target Higher Plants

Risk assessment for Terrestrial Non-Target Higher Plants

CP 10.6.1 Summary of screening data

CP 10.6.2 Testing on non-target plants

CP 10.6.3 Extended laboratory studies on non-target plants

CP 10.6.4 Semi-field and field tests on non-target plants

CP 10.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

Risk assessment for Other Terrestrial Organisms (Flora and Fauna)

CP 10.8 Monitoring Data