SULFONYLUREA HERBICIDES RISK ASSESSMENT ON BIOLOGICAL SYSTEM MODEL

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Abstract

This paper presents the obtained results regarding the environmental risk assessment for chlorsulfuron formulated as WG and WP and mixture between chlorsulfuron and tribenuronmetil in the same formulation. For testing it was used a biological system model in compliance with ISO 17025, respectively green algae. The main objective of our study was to maintain the RENAR accreditation for toxicity tests on green algae.
In the same time, for maintaining ISO 17025 accreditation they were performed management analyses, audits and corrective actions to settle nonconformities.
As result the Ecotoxicology Laboratory has maintained the RENAR accreditation for the above mentioned tests.

KEY WORDS: ecotoxicology, sulfonylureas, toxicity, alga

INTRODUCTION

The work has been performed in the framework of the „Research-Development" Program, accreditation / maintenance of the ISO 17025 accreditation being very important for our work giving confidence in the impartiality, independence and technical competence of accredited bodies working in the field of conformity assessment. Also, the accreditation / maintainance our accreditation it recognizes our competence in the work we develop in the field of ecotoxicology

The procedures applied in the laboratory are descriptions of the standard requirements or descriptions of laboratory tests which we performed. For each of the procedures they are specified the purpose, field of application, reference documents, terms and abbreviations, responsibilities and authority in the laboratory, describing activities, annexes and registrations.

MATERIAL AND METHOD

After the pre-evaluation audits and evaluation conducted by RENAR at our laboratory, accreditation certificate was obtained for determination of acute toxicity tests with fish, daphnia and algae.

In order to maintain accreditation it has been conducted a surveillance audit, the evaluation report prepared by the accreditation body confirmed that the ecotoxicology laboratory maintains and improves „Quality management system" according to SR EN ISO 17025/2005 and therefore we may perform accredited tests.

It was made also a review of the specific procedures in order to improve the activity of the laboratory, also to maintain and to improve the "Quality Management System" according to SR EN ISO 17025:2005.

They have been performed tests using accreditated specific procedures and « Quality Management System » according to SR EN ISO 17025/2005 with the object of environment risk assessment on algae for sulfonylurea herbicides, irrespectively clorsulfuron formulated as WG and WP and mixture between clorsulfuron with tribenuronmetil in the same formulation.

1. Purpose of the test

Determination of the effects of a substance on the growth of freshwater microalgae.
We use microalgae in aquatic toxicity testing because the following advantages:

- Very high sensitivity to definite categories of toxics, e.g. herbicides;
- Good representative of the lower level of the trophic chain in aquatic compartments;
- In spite of the relatively brief test duration (72 h), they are assessed the effects over several generations (chronic);
- Ease of culturing in the laboratory;
- If are used non-attached unicellular algae, direct counting is possible and the sedimentation issue is limited.
- All operations that involve the opening of the culture containers they are carried out under the hood with laminar flow in order to guarantee the sterility and the axenicity of the cultures themselves.
- In order to avoid the contamination of the cultures, all the glassware used for the maintenance of algal population and in the growth inhibition studies must be carefully cleaned, sterilized and properly stored.

2. Definitions

- *cell concentrations* is the number of cells per ml;
- *growth* is the increase in cell concentration per unit of time;
- *growth rate* is the increase in cell concentration per unit of time;
- EC_{50} is that concentration of test substance under examination causing a reduction of 50% of algal growth rate compared to the control, within the required period of exposure.
- *NOEC* (no observed effect concentration) the highest concentration tested at which no significant growth inhibition is noticed, as regards the control.

3. *Selection of species*

For plant protection products environment risk assessment on algae it was used an international guideline, OECD 201 /2006, EN ISO 8692: 2004 Water quality- Freshwater algal growth inhibition test with unicellular green algae and EN ISO 17025:2005, General requirements for the competence of testing and calibration laboratories. These standards recommend test species: *Pseudokirchneriella subcapitata* sin *Selenastrum capricornutum*, *Scenedesmus subspicatus* or *Chlorella vulgaris*

In our tests we used certificated algae from Culture Collection of Autotrophic Organisms (CCALA) Institute of Botany, Academy of Sciences of the Czech Republic.

4. Choosing the test concentrations

- Chose a range of concentrations based on available data (literature, data base) or preliminary test;
- Full (or definitive) test: 5 concentrations at logarithmic intervals and differing by a constant factor not exceeding 2.2. Ideally, the chosen range should provide at least one concentration that gives 0% effect and one that gives 100% effect;
- Limit test: 100 mg/l substance or product;

5. Preparation of inoculum cultures

In order to adapt the test alga to the test conditions and ensure that the algae are in the exponential growth phase when used to inoculate the test solutions, an inoculum culture in the test medium it is prepared 2-4 days before the start of the test. Incubate the inoculum culture under the same conditions as the test cultures.

6. Test conditions

Temperature: 23 ± 2 °C (for *S. capricornutum*);
Light: continuous (light intensity within the range of 6000–1000 Lux);
Test medium: reconstituted water, OECD medium;
pH of the test water: 8.3 at the beginning; it should not vary more than 1.5 units during a single test
Test duration: 72 hours;

Exposure regime: static and continuous or manual shaking

7. Monitoring of test parameters

pH: measured at the beginning and end of the test (one replicate for each test concentration and the control);

Ambient temperature: data logger or manual measure once per day;

8. Conditions for the validity of the test

A test is not acceptable if the cell density in the control cultures didn't increase by a factor of at least 16 within the test period;

The coefficient of daily growth rates variation in the control cultures must not exceed 35%;

9. Performance of the test

It was used the accreditated specific procedure for algae - Growth Inhibition Test. The system response is the reduction of growth of algal populations exposed to the test substance. The response is evaluated as a function of the exposure concentration in comparison with the average growth of replicate, unexposed control cultures. The culture flasks were shaken and placed in the growth chamber. During the test it was necessary to keep the algae in suspension and to facilitate transfer of CO_2. All the test conditions were registred.

The cell concentration in each flask (4 or 5 concentrations, each in 3 replicates) was determined at 24; 48 and 72 hours after the start of the test.

Growth inhibition is quantified from measurements of the algal biomass as function of time. Algal biomass is defined as the dry weight per volume, e.g. mg algae/litre test solution. However, dry weight is difficult to measure and therefore surrogate parameters are used, respectively cell counts. The cells were counting using a Thoma chamber. The measured cell concentrations in the test cultures and controls were tabulated together with the concentrations of the test substance and the times of measurements. To determine the concentration effect we used the comparison of areas under the growth curves

The herbicides used for tests were from sulfonylurea class which represent a major advance in global crop protection technology and have revolutionized weed control by introducing a unique mode of action. These compounds interfere with a key enzyme required for weed cell growth- acetolactate synthase. The toxicity tests were done with chlorsulfuron formulated like WG and WP and mixture between chlorsulfuron with tribenuronmetil in the same formulation. For testing it were used a biological system model in compliance with ISO 17025, respectively green algae- *Pseudokirchneriella subcapitata* sin. *Selenastrum capricornutum*

RESULTS

The test report has include informations about test substance, test organism, test conditions, concentrations tested and cell concentration for each flask at each measuring point and EC_{50} value and if it is possible NOEC.

Regarding to our tests, the available data suggest sulfonylureas, like many other herbicides, are much more toxic to aquatic plants than to aquatic animals (C. Sabater. J.M. Carrasco, 1997). Our results demonstrated these data and, in the same time, that a substance formulated like WP (wettable powder) is more toxic that the same substance formulated like WG (wettable granules).

The lowest EC_{50} value was for the mixture between chlorsulfuron 37,5% and tribenuron metil 37,5% formulated like WP: 1, 9798 mg sa/l. For the same mixture formulated like WG, EC_{50} value was 16,26 mg s.a/l.

The sensitivity of algae were different in the tests with chlorsulfuron, which is not so toxic like tribenuron metil (C. Sabater. J.M. Carrasco, 1997). So, EC_{50} values were 57,52 mg sa/l for WG formulation and 20,309 mg sa/l for WP formulation. These data demonstrated, once more, that WP formulation is more toxic than WG formulation, for the same substance.

The figures 1-4 ilustrate the growth curve for the four products tested. These graphics show that all the tests fulfill validity conditions, respectively the cell density in the control cultures has increased by a factor of at least 16 within the test period of observations.

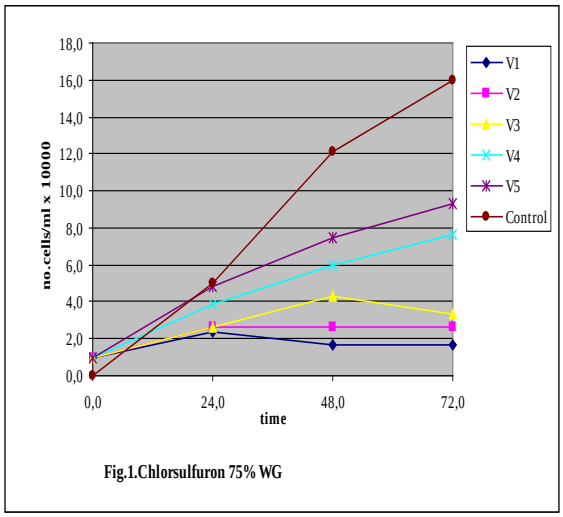

Fig.1.Chlorsulfuron 75% WG

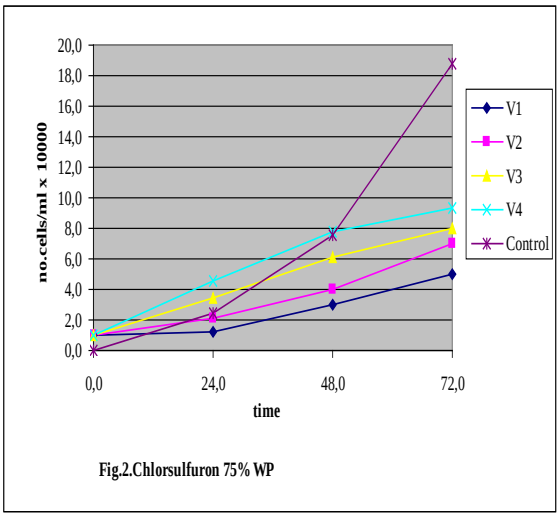

Fig.2.Chlorsulfuron 75% WP

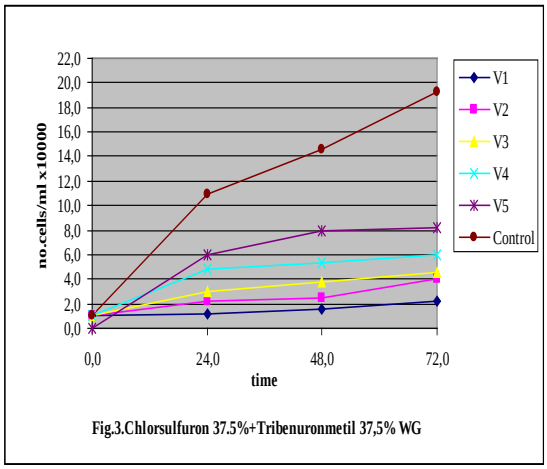

Fig.3.Chlorsulfuron 37.5%+Tribenuronmetil 37,5% WG

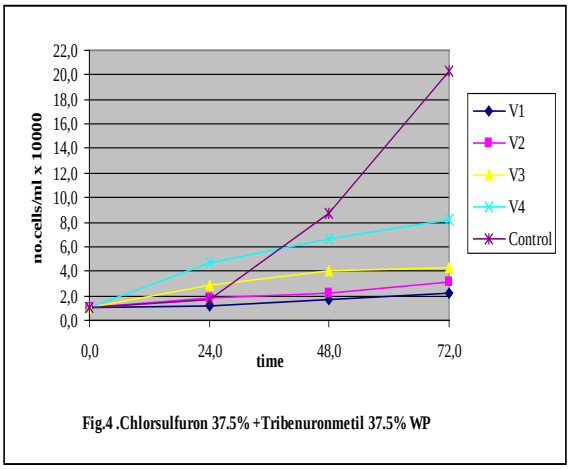

Fig.4 .Chlorsulfuron 37.5% +Tribenuronmetil 37.5% WP

CONCLUSIONS

The main objective of our study was maintaining the RENAR accreditation for toxicity tests on green algae. Conclusions of our the work was that even if the herbicides having a high ecological effciency, respectively low toxicity for fish and daphnia, they have a high toxicity for alga.

In the same time, for maintaining ISO 17025 accreditation they have been performed management analyses, audits and corrective actions to settle nonconformities.

As result the Ecotoxicology Laboratory mainteined the RENAR accreditation for the mentioned tests.

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