Canal de Isabel II

BOOKLETS

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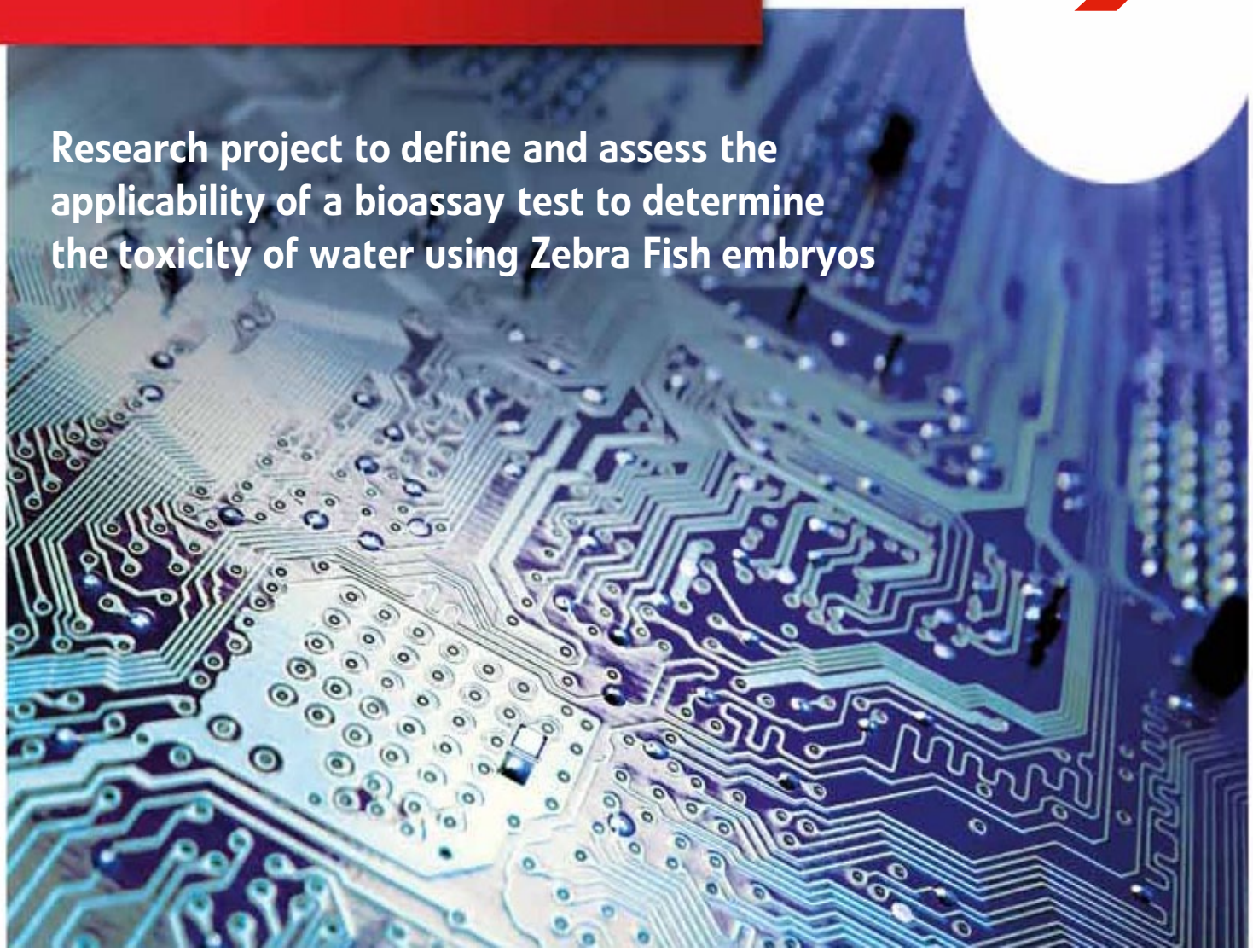
9

Research project to define and assess the applicability of a bioassay test to determine the toxicity of water using Zebra Fish embryos



Authors
J. Iñaki Urrutia Gutiérrez
Joaquín Guinea López
Juan F. Rodríguez Plaza
Paloma Acebo País

Project Direction
J. Iñaki Urrutia Gutiérrez
Rafael Heredero Rodríguez

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Introduction

Canal de Isabel II's Research, Development & Innovation Booklets form part of the company's Knowledge Management Strategy and of the development involved in the Research, Development and Innovation Plan.

These Booklets represent an element for diffusion of projects and initiatives that are developed and sponsored by Canal de Isabel II for innovation in those areas related with water service in the urban environment.

A series of different problems that have been undertaken in each project are put forward in the Booklets, along with the results that have been obtained. The intention behind their diffusion by means of these publications is to share the experiences and knowledge that has been acquired with the entire water services sector, with the scientific community and with all those working on investigation and innovation tasks. What is aimed with the publication of these Booklets is to contribute to improvement and efficiency in water management and, consequently, in the quality of service that is provided to the citizens.

The R&D&I booklets published to date are as shown below by their titles in the following table.

Collection Number	Research, Development and Innovation Booklets published
1	Transferences of Water Rights between Urban and Agrarian Demands. The case of the Community of Madrid
2	Identification of Hydrometeorological Runs and Tendencies within the scope of the Canal de Isabel II system
3	Contribution of Canal de Isabel II to the International Demand Management Project (IDMF)
4	Micro-components and Explanatory Factors on Domestic Water Consumption in the Comunidad de Madrid
5	Virtual Water and Hydrological footprint in the Comunidad de Madrid
6	Study on the saving potential of water for residential uses in the Comunidad de Madrid
7	Potentials of efficiency in using dishwashers
8	Accuracy in the measurement of individual water consumption in the Madrid Region

Project Outline

Project title	Research Project to define and assess the applicability of a Bioassay Test to determine the toxicity of water using Zebra Fish embryos (*Danio rerio*)
Research line	Drinking Water and Health
Canal de Isabel II units involved	Water Quality Deputy Direction; Research, Development and Innovation Deputy Direction
External participation	ZF Biolabs
Aim and justification of the project	In compliance with Royal Decree 140/2003, which regulates the quality of the drinking water that is supplied in Spain, the Water Quality Deputy Direction of Canal de Isabel II determines the physical, chemical, biological and radioactive parameters of the water that it manages. However, the regulations currently in force do not include the toxicological aspects. The early detection of toxic phenomena, especially Cyanophytes and Cyanotoxins and their control, is one of the water quality objectives set by suppliers such as Canal de Isabel II, hence the main aim of this project, which is to be able to have at its disposal a quick, effective and efficient test, which combines maximum reliability and minimum cost in time and resources, with a view to detecting toxicity in water as soon as it appears.
State of the art contribution	The research work for "Defining and evaluating the applicability of a Bioassay Test to establish the toxicity of water using Zebra Fish embryos (*Danio rerio*)" is a new line in experimentation, which has certain advantages over laboratory tests conducted with animals, because of its simplicity, transparency and the genetic similarities between this specie of fish and human beings. The aim is to detect abnormalities in the first cellular divisions of the Zebra Fish embryo, brought about by a variety of toxic substances. The embryo sensitivity is at maximum at this stage of development (blastula, 0.75-3.5 hpf). A series of FTZ protocols (*Fast Toxicity Zebra Fish*) have been fully developed that quickly detect the presence of toxicity in the water. Besides, certain more specific protocols have also been carried out for detecting hepatotoxicity and neurotoxicity. A line of research has been initiated involving the creation of a genetically modified Zebra Fish that is sensitive to the presence of hepatotoxins.
Project development summary and milestones	The research work has been made more difficult owing to a considerable lack of algal bloom from toxic Cyanobacteria –*Microcystins*-. Laboratory samples were prepared by drug adulterating the natural samples with toxic compounds that are harmful to human health. A total of 223 samples were analysed, taken from reservoirs and drinking water treatment plants managed by Canal de Isabel II. The toxicity test (FTZ), applied to the raw waters of the Canal de Isabel II does not follow patterns that are comparable with the physicochemical and biological laboratory analysis. The statistical processing conducted after the analysis reveals a toxicological potential for a 75% or 95% likelihood; 75% being indicative of toxicity, although not final; 95% indicates that toxicological potential is highly likely.
Obtained results summary	A rapid FTZ (*Fast Toxicity Zebra Fish*) test has been developed to detect after 1.5 hours with respect to whether or not there is toxicity in the water. A hepatoxicity test has also been worked out able to evaluate the LC5 and LC50 of the hepatotoxin in 6 days after the fertilization of Zebra Fish embryos. A neurotoxicity test has likewise been accomplished that assess the LC5 and LC50 of the hepatotoxin 20 hours after the embryo fertilization.
Research lines open for continuing the work	The biotechnological applications (*Recombinant Zebra Fish*) are a new tool for controlling drinking water quality, with a wide range of possibilities for detecting toxicological effects. After selecting the gene, whose expression is activated in the presence of a compound that is harmful to hepatocells, the gene is recombined with a green phosphorescent protein, in such a way that the embryo will radiate green fluorescent light when it grows in the presence of a hepatotoxic compound.

Executive Summary

Royal Decree 140/2003, dated 7th February, which sets out the health criteria for the quality of water to be used for human consumption, regulates the quality for the supply of drinking water in Spain. The Water Quality Deputy Direction, in compliance with this legislation, determines physical, chemical, biological and radioactive parameters; the regulations currently in force do not include toxicological aspects.

During the course of 2006 and 2007, the period when this project was developed, no cases were observed in the reservoirs or drinking water treatment plants (hereinafter ETAPs) of non-compliance with the regulations in force. From a hydrological perspective, 2006 was a dry year, which in general is conducive to causing deterioration in the quality of the water stored in reservoirs.

A research programme was prepared with a view to comparing the information concerning the analytical results collected in accordance with Royal Decree 140/2003, with the toxicity bioassay tests. The idea of the research work is to establish and assess the applicability of a Bioassay test to determine water toxicity using Zebra Fish embryos, which when compared to other tests conducted with animals, proves to be much simpler and transparent; the Zebra Fish also being genetically similar to human beings; this research work has being conducted jointly with the Comunidad de Madrid and the ZF-Biolabs biotechnological research laboratory.

During the course of this project a rapid toxicity test (FTX) has been perfected in such a way that, in a short period of time (1.5 hours), information is available about potential water toxicity. The method for extracting the toxins by sonication proved effective when it came to obtaining aqueous solutions from the reservoir samples containing algae. In view of the possibility that chemical components would be dissolved in the water that had nothing to do with the components released during the extraction process, the extract was resuspended directly into the filtered water, and both elements were tested together.

A hepatotoxicity test based upon Zebra Fish embryos has been developed and perfected, in such a way that it is possible to evaluate the LC5 and LC50 of the hepatotoxin, in a period of 6 days after fertilisation.

A neurotoxicity test has been developed and perfected, in such a way that it is possible to evaluate the LC5 and LC50 of the cyanotoxin in the Zebra Fish embryos, in a period of 20 hours after fertilisation.

A total of 223 samples were analysed, with a view to making comparisons and conducting the required bioassay tests. For a considerable period of time while the tests were being performed, the lack of situations or types of water that could give positive results in the tests used, meant that it was necessary to prepare a variety of samples, in some cases not using the raw water, by adulterating them with compounds that according to the product safety files are toxic and harmful to human health.

A statistical method was applied to the results of the samples; this method shows the toxicological potential to a large extent, for a 75 or a 95% probability. When a sample was available to carry out repetitions, an estimation was made of the total toxicity, which is a more accurate value because a greater number of cases were available. That is why the samples whose positive toxicological potential is 95% and those that reveal toxicity in the total, are considered to be significantly toxic. When the value is positive at 75% where toxicological potential is concerned, this is considered to be indicative, but not definitive.

After the FTZ test was conducted, positive results were obtained in 38 cases, of which 35 yielded positive toxicological potential (12 yielded positive results, at 95 and 75% and the remaining 23 only at 75%, the indicative but not definitive value). The rest were samples with a negative toxicological potential, but with toxicity in the total, so they are regarded as toxic.

These positive results (38) found by ZF-Biolabs using the FTZ test were crossed with the regular analyses that is applied by the Laboratory belonging to the Water Quality Deputy Direction, in compliance with Royal Decree 140/2003, in order to seek potential interrelationships between the toxicity tests and any possible water quality incidents in the reservoirs and the drinking water treatment plants (ETAPs). On 4 occasions the expected positive results coincided with the FTZ test (2 cases involving the death of carp in Pedrezuela, another case in the ETAP for that reservoir, with algal bloom, and the fourth one in Valmayor, also because of the development of cyanophytes). No analytical coincidence was found for the toxicity in the rest of the FTZ positive results, and on other occasions, with cyanophyte blooms, negative results were obtained, although not all the test results have yet been analysed.

It can be deduced from the results obtained that the toxicity tests (FTZ) applied to Canal de Isabel II's raw waters, do not follow patterns that are comparable with the physicochemical and biological analyses conducted in the laboratory. This could be due either to the different nature of the type of test, or to the synergic effects or even to the potential contrasts and side effects of many of the products that can be found inside the cells of algae, or it could be caused by the dumping of waste or solutions that can occur in the natural environment. The absence of large quantities of "algal bloom" from toxic cyanobacteria throughout most of the study period also made it extremely difficult to achieve this objective, because of a lack of raw material, i.e. *microcystins*, in sufficient quantities to evaluate the natural toxicity of the water.

With respect to the special samples that were used to compare the correctness of the bioassay tests, it has been observed that there were a lot of cases where the expected effects did not take place at the beginning. Only one positive case (with HAP) was detected out of 5 doped up samples. However, when the samples were duplicated, results were yielded that coincided between the four pairs (one positive case, and the rest with negative toxicity).

These differences might be explained by a variety of factors, the first of these being that on a large number of occasions, the majority, work was done with the "particulated" contents of the sample, that is to say, with the extract from the sample, mainly composed of phytoplankton, but rejecting the dissolved part. Having let the dissolved content of the sample pass through, could undoubtedly have affected the expected results, because most of the compounds added, which were toxic, were dissolved and rejected, so the extract used did not affect the way the Zebra Fish embryos developed.

The neurotoxicity and hepatotoxicity tests did not yield significant results, many of the samples being dubious, perhaps due to a lack of samples with positive toxicity.

As far as the use of recombinant zebra fish to test toxicity is concerned, it must be pointed out that, of the 5 genes selected for the study, the γ-glutamyl transferase gene, could be a suitable candidate for the creation of a genetically modified Zebra Fish that is able to detect toxic compounds in water, even if it has not been sufficiently tested with *microcystins*. This genetically modified fish would provide the promoter of the γ-glutamyl transferase gene fused before the gene that encodes the fluorescent green protein. The expression of this gene is greater when the embryos are incubated in the presence of the toxic compound as from the first hours of life, so genetically modified Zebra Fish embryos would be used to detect toxic compounds in the water, instead of individual adults.

A variety of articles have been written and papers presented concerning the project and the results obtained, so that the research work done can be examined and publicised in a suitable scientific environment.

www.canalgestion.es/galeria_ficheros/compromiso-social/publicaciones/Cuaderno9_I+D+i.pdf

Canal de Isabel II

www.cyii.es

Canal de Isabel II
Santa Engracia, 125
28003 Madrid (Spain)